J Radioanal Nucl Chem (2010) 285:621–626
DOI 10.1007/s10967-010-0578-5

Development of metallocomplex amino acids synthons for the asymmetric preparation of α-amino acids by stereoselective introduction of a side chain. Evaluation of the model asymmetric preparation of alanine and β-^{13}C monolabelled α-aminoisobutyric acid

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Received: 14 April 2010 / Published online: 1 May 2010

Abstract In this communication the evaluation of eleven new metallocomplex alanine synthons bearing C_2-symmetric benzyl groups with electron-donating and electron-withdrawing substituents is described. α-Methylated glycine synthons (alanine complexes) were evaluated alongside alanine synthons in order to obtain a deeper understanding of the relationship between their structures and stereochemistry of monoalkylated products and to choose several candidates for their further tests for stereospecific preparation of 6-[^{18}F]FDOPA. Glycine-derived analogues of the complexes 3–5 are the best candidates for the development of a 6-[^{18}F]FDOPA preparation procedure. In the model epimerisation reaction they demonstrated the best performance, much better compared to the previously described compound 2. Complexes 3, 5 and 8 are the best in asymmetric preparation of β-^{13}C monolabelled α-aminoisobutyric acid. They have to be tested in the preparation of α-methyl amino acids like 6-[^{18}F]-α-methylDOPA and 2-[^{18}F]-α-methyltyrosine.

Keywords Amino acids · 6-[^{18}F]FDOPA · Stereoselectivity · Nickel · PET · Schiff bases

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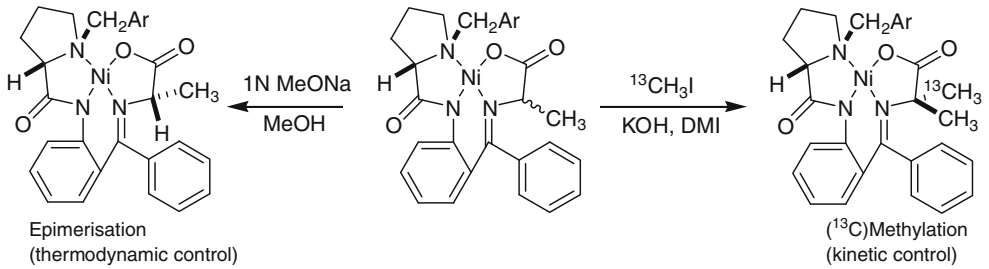
Introduction

PET diagnostics using radiolabelled amino acids is an emerging branch of nuclear medicine [1–6]. This includes visualisation and grading of brain, neuroendocrine and prostata tumours, measurement of protein synthesis rate in tumour cells, quantitative in vivo measurement of dopamine and serotonin metabolism in brain. Development of clinical applications is limited by complexicity of robotic devices necessary for multi-step preparation of the enantiomerically pure amino acids. Robust and reliable approaches which do not require sophisticated separation of radiolabelled intermediates have to be created for everyday clinical routine. Efficient asymmetric synthesis of carbon-11 labelled amino acids based on chiral nickel complexes [7–10] was suggested [11]. Current approach using well-developed chiral auxiliary (*S*)-*N*-(2-benzoylphenyl)-1-benzylpyrrolidine-2-carboxamide (BPB) in nickel(II) complex of its Schiff base with glycine allows easy preparation of

[^{18}F]FET with 94–97% *e.e.* with no need for separation of diastereomers of alkylated complexes [6]. Preparation of 6-[^{18}F]FDOPA using the same starting nickel complex gives only 77 ± 5% *e.e.* without separation of diastereomers of alkylated complexes while the same preparation of *β*-[^{11}C]DOPA leads to 92–99% *e.e.* [12, 13]. Preparation of α-methylDOPA or α-methyltyrosine labelled with carbon-11 or fluorine-18 requires formation of quaternary chiral centre, stereochemistry of which is controlled kinetically. Kinetic control is much less efficient then thermodynamic one, thus diastereomeric excess of complexes of α-methyl amino acids is inferior [14]. Enhancement of stereodivergent power in both thermodynamically and kinetically controlled alkylation reaction is the biggest priority in development of new metallocomplex tools for the preparation of PET amino acids. Stoichiometric asymmetric syntheses are better suited for the preparation of PET radiotracers then the catalytic ones. The synthetic pathway via Ni(II) complexes brings important advantages for nanomolar-scale radiosyntheses, like stability of the starting compounds during prolonged storage on air at ambient temperature, no need for very strong bases for generation of the intermediate carbanion and easy UV-detection of reddish alkylation products during HPLC separation of reaction mixtures, considerable attention was paid to improvement of the stereochemical output of alkylation of the complexes [15–18]. Our approach is based on disclosure of factors influencing intramolecular interactions in the complexes and shielding of one side of (pro)chiral centre. Data from X-ray structure determinations and mapping of deformation electron density, NMR studies of conformations of the complexes in solutions and solid state, ab initio MP2 modeling followed by topological analysis led to a lead structure: a complex carrying a C_2-symmetric benzyl

Table 1 Methylation and epimerisation of alanine complexes



	Substituents of the benzyl group			Epimerisation		(13C)Methylation	
No	*Ortho*-substituents	*Meta*-substituents	*Para*-substituent	*d.e.* (%)[a]	Starting complex	*d.e.* (%)[b]	Alkylated complex
1	H	H	H	75	1a	39	1b
2	CH_3	H	CH_3	97[c]	2a	66[c]	2b
3	CH_3	CH_3	CH_3	>99[d]	3a	75	3b
4		9-Anthryl		>99[d]	4a	69	4b
5	H	*tert*-Bu	H	>99[d]	5a	78	5b
6	H	OCH_3	H	91	6a	73	6b
7	H	CH_3	H	80	7a	60	7b
8	H	CF_3	H	94[e]	8a	80[e]	8b
9	F	H	H	89	9a	44	9b
10	H	H	*tert*-Bu	78	10a	44	10b
11	H	H	OCH_3	86	11a	42	11b
12	H	H	OCF_3	91	12a	53	12b
13	H	H	CF_3	89	13a	52	13b

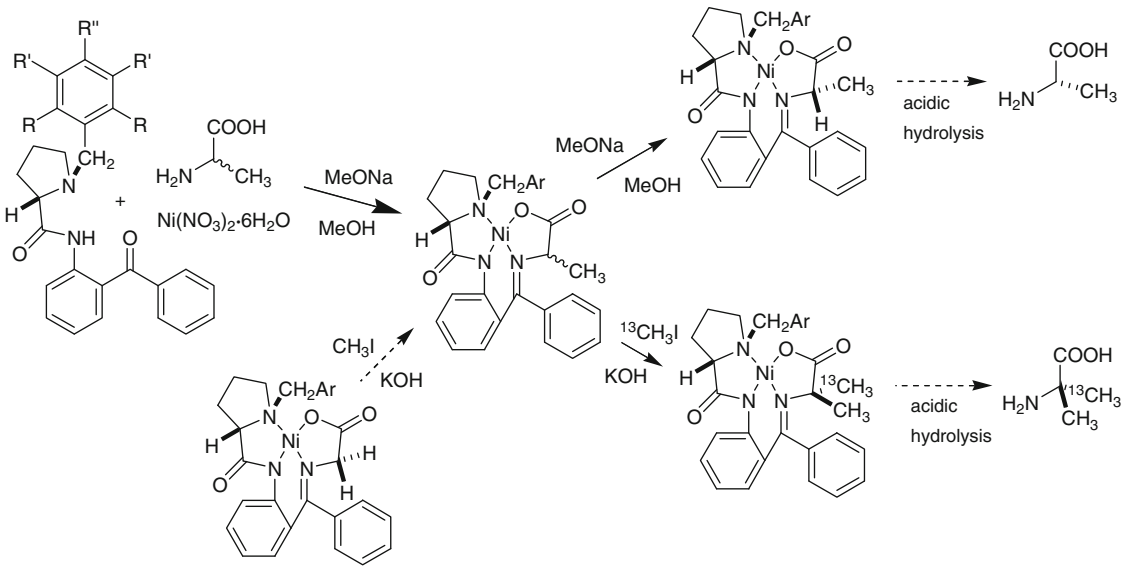
[a] Based on ratio of integral intensities of CH_2Ar quadruplet or CH_3 doublet of the minor isomer to corresponding peaks of ^{1}H–^{13}C satellites of the major isomer in ^{1}H-NMR spectra of reaction mixtures

[b] Based on measurement of integral intensities of methyl group peaks in ^{13}C-NMR spectra recorded with relaxation time 20 s. Calculated according to (1)

[c] Data from Ref. [23]

[d] No signal of the minor diastereomer was found in the ^{1}H-NMR spectrum of the reaction mixture

[e] Preparation of this alanine complex was described earlier, but no epimerisation or (^{13}C)methylation measurements were done [18]



Scheme 1 Asymmetric preparation of alanine and β-^{13}C monolabelled α-aminoisobutyric acid

group with electron-donating groups in both *ortho*-positions [19–22]. The first structures prepared were Ni(II) complexes of modified BPB and glycine or α-alanine. Both structures were tested in methylation reactions in order to create the most challenging conditions by use of a small electrophile molecule. Methylation of the glycine derivative followed by epimerisation was practically stereospecific [23, 24]. (^{13}C)Methylation of the alanine derivative led to 66% *d.e.* (due to kinetic control of stereochemistry) [23]. An analogous glycine synthons carrying a chlorine substituent in *ortho*-position of the benzyl group or two chlorine substituents in *meta*- and *para*-position of the benzyl group allowed preparation of 6-[^{18}F]FDOPA with ~90% *d.e.* while the similar alanine synthons failed to deliver high asymmetric induction in the preparation of 6-[^{18}F]-α-methylDOPA. (R.N. Krasikova, Y.N. Belokon, unpublished results) In this communication the evaluation of eleven new alanine synthons bearing C_2-symmetric benzyl groups[1] with electron-donating and electron-withdrawing substituents is described (Table 1; Scheme 1).

Results and discussion

The optimisation of the complex structures required taking into account both steric and electronic effects of substituents. The first step was to increase the steric hindrance of the substituted benzyl group by introduction of bigger (and/or more) substituents. In the case of electron-donor substituents this should also increase electron density donated to the aromatic ring, thus increasing polarisation of its electron cloud towards the positively charged nickel atom, followed by stronger electrostatic interactions between the aromatic ring and nickel [20]. Electron-withdrawal substituents like –F and –CF_3 reduce electron density on the aromatic ring, but at the same time they can form relatively strong intramolecular C–F…H hydrogen bonds with the distant parts of the tetradentate ligand. Such bonds may favour the formation of one of the diastereomers of the complexes in alkylation reaction. With the exception of three complexes carrying *ortho*-substituents: 3a bearing pentamethylbenzyl group, 4a bearing (9-anthracenyl)methyl group and 9a with 2,6-difluorobenzyl group; all other newly synthesised compounds have *meta*-disubstituted or *para*-monosubstituted benzyl groups in order to compare the influence of the same substituents in *ortho*-, *meta*- and *para*-positions. It is possible to introduce very bulky *tert*-butyl substituents to *para*- and *meta*-positions only. Syntheses and evaluation of complexes with electron-withdrawing fluoro or trifluoromethyl substituents was necessary for separation of the steric and electronic influence of the substituents and the influence of intramolecular C–F…H hydrogen bonds. There was a published report about highly stereoselective alkylation of a complex carrying one chlorine substituent in *ortho*-position where similar factors may account for high stereoselectivity [18].

Synthesis of complexes 3a and 3b, where the leads structures 2a and 2b were modified by addition of two electron-donating *meta*-methyl substituents into the benzyl group, supported the initial hypothesis—both thermodynamically controlled epimerisation and kinetically controlled (^{13}C)methylation run more efficiently.

[1] Symmetry of all used benzyl groups was higher (C_{2v}), C_2-symmetry is a minimum requirement for elimination of influence of the group rotation to intramolecular interactions.

Diastereomeric excess of methylation increased from 66 to 75%. Complex 7a bearing only two *meta*-methyl substituents is not so efficient as complex 3a, probably due to an important steric role played by the *ortho*-methyl substituents and lower donation of electron density by only two substituents. The importance of steric effect brought by *ortho*-substituents is confirmed by preparation of complexes 9a and 9b, where smaller planar –CH=C– substituents in the *ortho*- and *meta*-positions are not as efficient as *ortho*-methyl substituents in complex 3. Compound 5a bears very bulky *tert*-Bu substituents in *meta*-positions. Its stereodiscriminative efficiency is even than complex 3a. Stronger repulsion between the (bulky) substituted benzyl group and the equatorial methyl group during epimerisation may also play a role. Electron-withdrawing CF_3 substituents in *meta*-positions of the benzyl group gave a very efficient synthon, probably due to formation of hydrogen bonds between fluorine atoms and the hydrogens on the benzophenone part of the ligand. This explanation is consistent with results observed with *para*-substituted complexes 10–13, where compounds carrying CF_3 or OCF_3 substituents over perform similar complexes with bulky *tert*-Bu or electron-rich OCH_3 substituents. Fluorine atoms in *ortho*-positions of the benzyl group improve the results of compound 9a compared to complex 1a. In solid state one of the fluorine atoms is situated in apical position of the complex (Fig. 1), thus confirming strong electrostatic interaction between this negatively charged atom and the positively charged nickel, similar to weaker intramolecular interactions observed in the glycine analogue of 1 [20]. This interaction is probably responsible for higher efficiency of epimerisation of this complex compared to its steric analogue 1a. Kinetically controlled (^{13}C)methylation is not very much affected by the interaction; this corresponds to quick rotation of the benzyl groups around their axis of symmetry in both 1 and 9 revealed by their NMR spectra in $CDCl_3$. Similar electrostatic interactions possibly play their role in behaviour of analogous complexes bearing *ortho*-Cl or poly-F substituents of benzyl group [18, 25]. X-ray structure determination of the complex 3a also revealed an apical placement of the pentamethylbenzyl group confirming similar, but more profound intramolecular interactions as previously described for the glycine analogue of the complex 1 (M. Fronc, J. Kožíšek, A. Popkov, unpublished results).

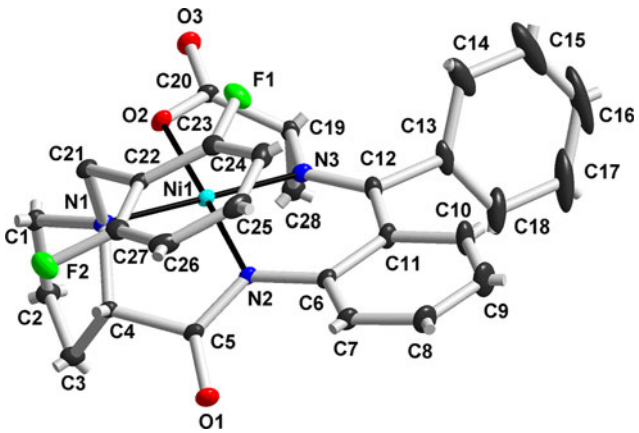


Fig. 1 X-ray structure of complex 9. Numbering scheme with atomic displacement ellipsoids drawn at 30% probability level. Hydrogen atoms omitted for clarity

Glycine-derived analogues of the complexes 3a–5a are the best candidates for the development of 6-[^{18}F]FDOPA preparation procedure. In the model epimerisation reaction they demonstrated the best performance, much better compared to the previously described compound 2a. Complexes 3a, 5a and 8a have to be tested in the preparation of 6-[^{18}F]-α-methylDOPA and 2-[^{18}F]-α-methyltyrosine. Their analogues derived from protected DOPA or tyrosine are the most promising candidates for the preparation of α-[^{11}C]methylDOPA and α-[^{11}C]methyltyrosine.

Experimental

General procedure for the template synthesis of the alanine complexes

2 M MeONa/MeOH (5 mL, 10 mmol) was added to a stirred suspension of a chiral auxilliary (0.65 mmol), alanine (195 mg, 1.3 mmol) and nickel nitrate hexahydrate (227 mg, 0.78 mmol) in dry MeOH (5 mL) under argon at 55 °C. After stirring at 55 °C for 30 min, the mixture was poured into 3% aqueous citric acid (150 mL), stirred and the resulting precipitate was filtered off and dried in air. The dry red precipitates (or red solidified oils) was purified by preparative TLC on silica gel eluted with chloroform followed by gel chromatography on Sephadex LH-20 (eluent toluene/methanol = 2:1) and characterised by ^{1}H-NMR, ^{13}C-NMR, ESI–MS spectra measured with high-resolution QqTOF analyzers providing information on the elemental composition of protonated complexes. Yields are 70–80%.

Mass spectra were measured on an orthogonal hybrid quadrupole time-of-flight (QqTOF) mass spectrometer fitted with electrospray ionization source (Bruker Daltonics, Bremen, Germany). The instrument was externally calibrated using ESI tunning mix before the measurement. The samples were dissolved in acetonitrile and analyzed by direct infusion at the flow rate of 3 μL/min. Interface parameters were set as follows: capillary voltage −4.5 kV, drying temperature 200 °C, the flow rate and pressure of nitrogen were 4 L/min and 0.4 bar, respectively. For the recording of exact masses, QqTOF data were acquired by

summation of 50,000 scans with 10 rolling averages. Protonated molecules $[M + H]^+$ and the adducts with alkali metal ions, such as $[M + Na]^+$ and $[M + K]^+$ were observed in the full scan positive-ion ESI mass spectra for all studied compounds which was in accordance with our previous work [26]. The elemental composition was confirmed based on the determination of accurate *m/z* values with mass accuracies better than 5 ppm.

Epimerisation of alanine complexes

Under argon the complex (0.1 mmol) was dissolved in 10 mL of MeOH. 2 N MeONa/MeOH was added in order to adjust the concentration of MeONa to 1 N. After 30 min the epimerisation mixture was poured into 5% aqueous citric acid (200 mL), stirred and extracted with CH_2Cl_2 (4 × 20 mL). The combined extracts were evaporated and directly used for determination of the ratio of the diastereomers by ^{1}H-NMR. Integral intensities of CH_2Ar quadruplet or CH_3 doublet of the minor isomer and corresponding peaks of ^{1}H–^{13}C satellites of the major isomer in ^{1}H-NMR spectra were applied for the determination of the ratios of diastereomers of alanine complexes (Table 1). The spectra were recorded with Bruker Avance 500 spectrometer (Bruker, Silberstreifen, Germany).

Methylation of alanine synthons by $^{13}CH_3I$

At 20 °C to a 0.05 M solution of a complex, an excess of KOH and a five fold excess of $^{13}CH_3I$ were added and the reaction mixture was stirred for 30 min under argon. The reaction mixture was poured into 3% aqueous citric acid, stirred and the resulting precipitate was filtered off and dried in air. The dry red precipitates (or red solidified oils) was purified by preparative TLC on silica gel eluted with chloroform followed by gel chromatography on Sephadex LH-20 (eluent toluene/methanol = 2:1). Yields are 20–60%.

Identical experimental conditions as for the mass spectra measurement of starting alanine complexes described above were used. The elemental composition was confirmed based on the presence of protonated molecules $[M + H]^+$ and the adducts with alkali metal ions in the full scan positive-ion ESI mass spectra. Moreover, ions corresponding to non-reacted starting alanine complexes were observed together with labelled compounds.

^{13}C-NMR spectra were applied for the determination of the ratios of diastereomers of complexes of β-^{13}C monolabelled α-aminoisobutyric acid in the reaction mixtures. The spectra were recorded with Bruker 500 spectrometer with 20 s intervals between pulses for complete relaxation. Diastereomeric excess calculations were based on the ratio of the integral intensities of the $^{13}CH_3$-signals in the ^{13}C

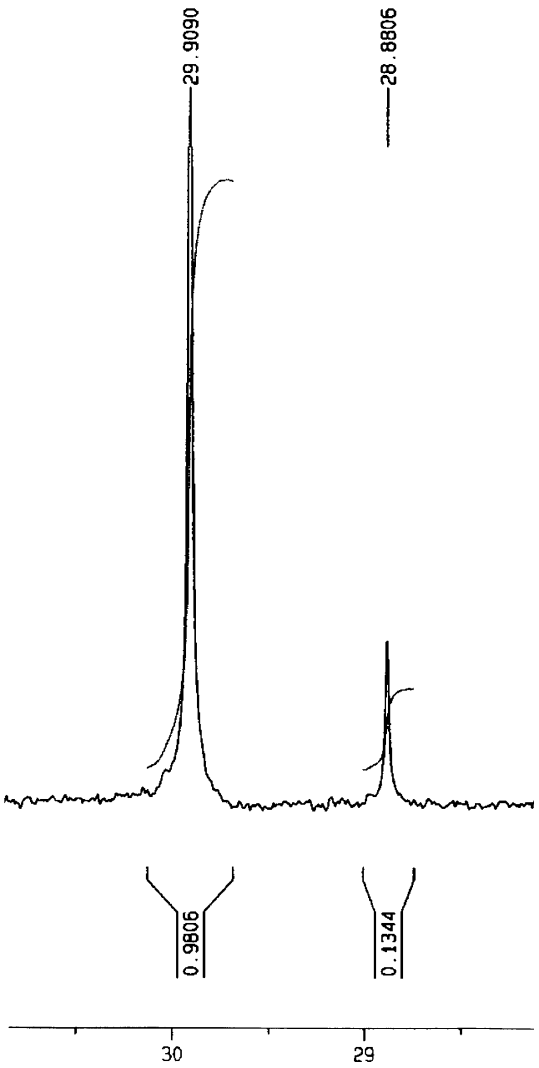


Fig. 2 A fragment of the ^{13}C-NMR spectrum of 6b. Signals of (^{13}C)methyl groups of the both diastereomers

NMR spectra of the mixtures of the diastereomers (Table 1; Fig. 2);

$$\text{D.e.} = 100 - 200 \cdot (a \cdot [S, R]/[S, S]^* - b)/(a - b) \cdot (1 + [S, R]/[S, S]^*) \quad (1)$$

where $[S, R]/[S, S]^*$ is the ratio of the integral intensities of the ^{13}C-signals of the diastereomers; ***a*** is the abundance of ^{13}C in starting $^{13}CH_3I$; ***b*** is the natural abundance of ^{13}C.

Conclusions

Newly prepared amino acids synthons 3–5 and 8 demonstrate a very high level of stereocontrol in both thermodynamically and kinetically controlled alkylation reactions. They are promising leads for further development of stereospecific synthons of [^{18}F]FET, 6-[^{18}F]FDOPA,

α-[^{11}C]methyl amino acids for PET and α-(^{13}C)methyl amino acids.

Acknowledgments The authors acknowledge the support of project grants MSM0021627501 (J. H. and M. N.), MSM0021627502 (R. J. and M. H.) and MSM6007665808 (A. P.) sponsored by the Ministry of Education, Youth and Sports of the Czech Republic.

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