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Research Article

A novel and efficient asymmetric synthesis of carbon-14 labeled (*S*, *S*)-2,7-di-boc-diamino[1,8-$^{14}C_2$]suberic acid

A. J. Villani*, D. Saunders, A. Y. L. Shu and J. R. Heys
GlaxoSmithKline Pharmaceuticals, Department of Process Strategy and Support, Isotope Chemistry Section, UW2830, King of Prussia, PA 19406, USA

Summary

Five hundred mCi of Potassium [^{14}C]cyanide at a specific activity of 51 mCi/mmol was used to diastereoselectively introduce the carbon-14 label into 1,6-hexanedial via a thermodynamically controlled asymmetric Strecker reaction using (*R*)-(-)-2-phenylglycinol as the chiral auxiliary. The expected and predominant (*R*, *S*/*S*, *R*) diastereomer (**2**) was separated by preparative normal phase HPLC. The chiral auxiliary was removed by oxidation with lead tetraacetate and the resulting benzylimino nitrile exhaustively hydrolyzed in hydrochloric acid to give (*S*, *S*)-2,7-diamino[1,8-$^{14}C_2$]suberic acid (**6**). Subsequent acylation with di-tert-butyldicarbonate gave the title compound (**1**) with a radiochemical purity of 95.5%, a specific activity of 113 mCi/mmol, and an enantiomeric purity of 95.5% e.e. To our knowledge this is the first report of the asymmetric Strecker methodology being applied to the synthesis of a carbon-14 labeled amino acid. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: asymmetric Strecker reaction; thermodynamic control; (*R*)-(-)-2-phenylglycinol chiral auxiliary; potassium [^{14}C]cyanide; carbon-14 labeled amino acid

*Correspondence to: A. J. Villani, GlaxoSmithKline Pharmaceuticals, Department of Process Strategy and Support, Isotope Chemistry Section, UW2830, King of Prussia, PA 19406, USA.

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Introduction

Peptides in which the disulfide linkage of the cystine unit has been replaced with the isosteric dicarba equivalent **1** have shown high biological activity and enhanced metabolic and chemical stability.[1] As a result, interest in the synthesis of **1** has gained much attention in the last few years.[2,3]

The peptide SK&F-107647 was selected from preliminary screens for further study because of its potency as a regulator of CSFs (colony stimulating factors) in certain cell lines.[4,5] To determine the safety of SK&F-107647 in preclinical species, whole body autoradiography (WBAR) and excretion studies in rats and dogs were needed using isotopically labeled peptide. Since the studies with tritium labeled peptide did not provide definitive data due to exchange with water *in vivo*, a high specific activity carbon-14 labeled analog of SK&F-107647 was requested with the carbon-14 located in the more metabolically stable part of the molecule, namely, the isosteric dicarba equivalent of cystine (Figure 1).

This necessitated the synthesis of carbon-14 labeled chiral (*S*, *S*) 2,7-Di-BOC-diaminosuberic acid (**1**, Figure 1) labeled at C1 and C8 described herein. Asterisks denote the positions of the carbon-14 labels.

Results and discussion

Of the various synthetic strategies available to us, the Strecker amino acid synthesis offered the most convenient and inexpensive experimental

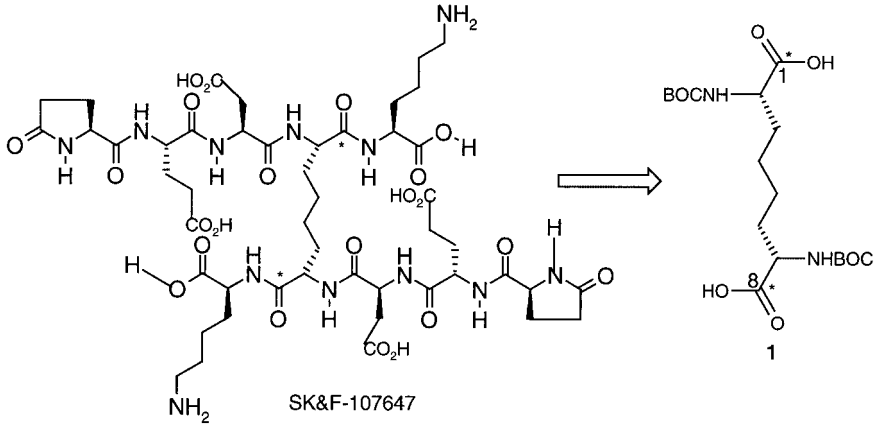


Figure 1. Structure of SK&F-107647 and 1

protocols for preparing carbon-14 labeled amino acids. The Strecker reaction has been known since 1850, and the first asymmetric Strecker reaction was reported in 1963 by Harada.[6]

The Strecker methodology we chose to follow relied greatly on the work of Ogura[7] (Figure 2) who achieved a high equilibrium ratio of **I**:**II** in a thermodynamically controlled 1,3-asymmetric induction using (*R*)-(-)-2-phenylglycinol as the chiral auxiliary. Thus, when the appropriate aldehyde is treated with (*R*)-(-)-2-phenylglycinol in the presence of KCN and $NaHSO_3$ in aqueous methanol an equilibrium is established. This equilibrium is believed to involve the intermediacy of the Schiff base **A**.[8]

We envisaged a similar equilibration in the novel case of a dialdehyde. Our results obtained with 1,6 hexanedial appear to be consistant with the above equilibrium hypothesis in that a diasteroselectivity of 3:1 was obtained in favor of the desired and equivalent ($R, S/S, R$) isomer (**2**). In addition to the use of a dialdehyde, other modifications of the Ogura procedure[7] were necessary in order to conform to the usual radiosynthetic requirements. In our case, this entailed making potassium [^{14}C] cyanide the limiting reagent.

The unstable 1,6-hexanedial (2 eq.) was generated *in situ* via the oxidation of cyclohexene[9] and then treated directly with 500 mCi (1.6 eq.) of potassium [^{14}C]cyanide in the presence of (*R*)-(-)-2-phenylglycinol, giving a 3:1 mixture of diastereomers **2** ($R, S/S, R$) and **3** ($R, S/R, R$) (meso), respectively. Very little of the ($R, R/R, R$) isomer (**4**) was produced. As expected, the ($R, S/S, R$) diastereomer (**2**) was shown to give **1** as determined by chiral capillary electrophoresis analysis. In non-isotopic trial runs, the above diastereomeric mixture was carried through to a mixture of enantiomers containing **1**, and then analyzed by chiral capillary electrophoresis versus reference standards for the (S, S), meso (S, R), and (R, R) isomers of **1**. The analysis showed the enantiomeric mixture to contain: 72% (S, S), 25% meso and 3% (R, R).

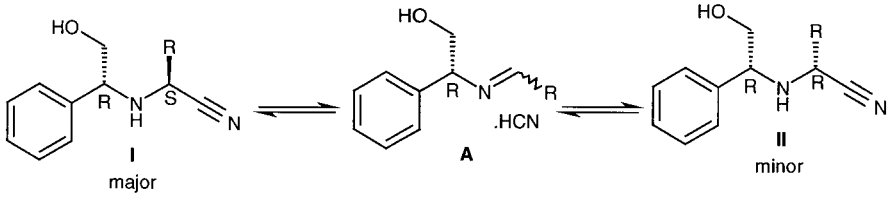
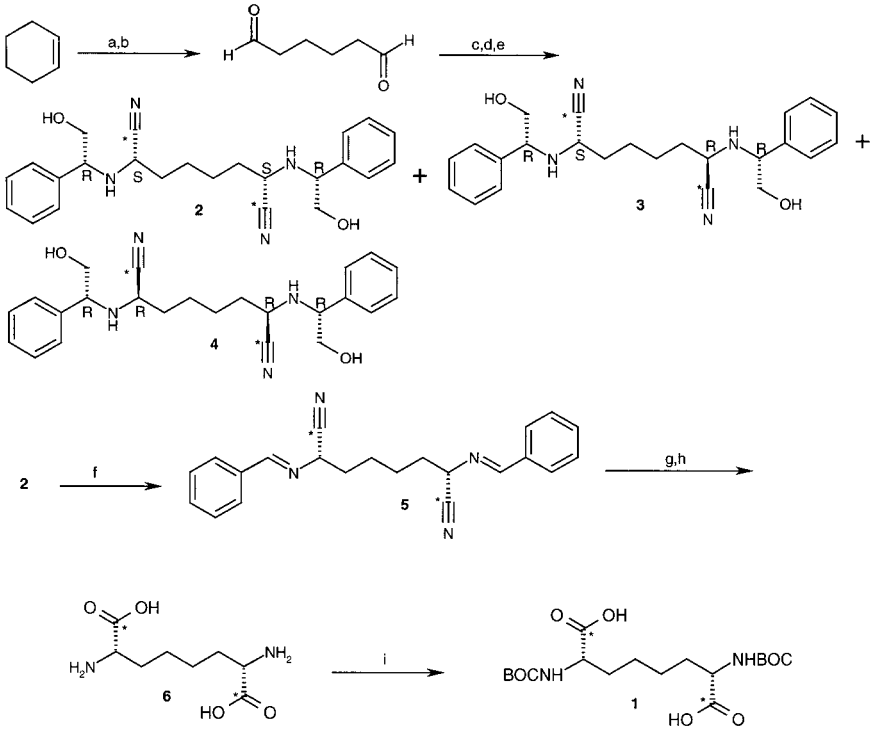


Figure 2. Equilibration of a-aminonitriles derived from (*R*)-(-)-2-phenylglycinol and aldehydes

The predominant and desired diastereomer **2** was separated by preparative normal phase HPLC in an overall yield of 29% from potassium [^{14}C]cyanide. Surprisingly, attempts to remove the chiral auxiliary with Pd-catalyzed hydrogenolysis[10] were not successful. However, we discovered that oxidation of **2** with 3 equivalent of lead tetraacetate[11] in methylene chloride at 0°C afforded the Schiff base **5** in an 86% radiochemical yield. Subsequent hydrolysis of the resulting crude **5** with 6 N HCl at ambient temperature, then with concentrated HCl at 95°C afforded the expected **6** in an 89% radiochemical yield. No racemization was detected by capillary GC.

The ensuing acylation of **6** with di-tert-butyldicarbonate gave a 54% radiochemical yield of **1** having a radiochemical purity of 95% (TLC), a specific activity of 113 mCi/mmol (MS), and an enantiomeric purity of 95.5% e.e. (GC) (Scheme 1).



Scheme 1. Synthesis of (*S*, *S*)-2,7-Di-BOC-diamino[1,8-$^{14}C_2$]suberic acid (1). Reagents and conditions: a. OsO_4(cat), b. $NaIO_4$, Et_2O/H_2O, rt; c. K^*CN, d. $NaHSO_4$, e. (*R*)-(-)-2-phenylglycinol, CH_3OH/H_2O, rt, 68 h; f. LTA, CH_2Cl_2, 0°C; g. 6N HCl, rt, h. cHCl, 95°C, 18 h; i. $(BOC)_2O$, pH 9, aq. CH_3CN, rt, 14 h

Compound **1** was successfully utilized in the synthesis SK&F-107647-[^{14}C] (Shu AYL, Heys JR. GSK *Unpublished Results* 1996).

Conclusion

We believe that the generality, mild conditions, convenience and efficiency of this methodology make it worthy of a place in the arsenal of carbon-14 chiral amino acid labeling techniques. Hanus[12] reported a racemic synthesis of very low specific activity carbon-14 labeled **6** via the alkylation of sodium diethylacetamido [2-^{14}C]malonate with 1,4-dibromobutane. Our method compares favorably to that of Hanus's in terms of overall radiochemical yield to **6**, and has the added advantage of using less expensive labeled starting materials.

To our knowledge, this is the first report of **1** being prepared labeled with carbon-14 via an asymmetric Strecker reaction, and the first example of Ogura's methodology being applied to the preparation of a chiral bis-amino acid.

Experimental section

General

Low-resolution mass spectra were obtained on a Finnegan 3625 instrument operating in the chemical ionization mode using ammonia as reagent gas for the determination of specific activity. Enantiomeric purities were obtained on Hewlett-Packard 5890 gas chromatograph using a Chirasil Val^{TM} 25 M × 0.25 mm × 0.16 um column, injector temperature of 250°C, detector temperature of 275°C, FID detector, linear velocity 40 cm/s, carrier gas He, makeup gas N_2, injection split 1.5:1, temperature profile: 90°C for 4 min then 4°C/min to 180°C for 20 min. Thin layer chromatograms were run on 5 × 20 cm, 250 um Merck and Analtech Silica Gel plates using a solvent system of 10:1:2 C_2H_5OH:H_2O:15 N NH_4OH except where noted in the preparation. Capillary electrophoresis was carried out on a fused silica, 50 cm (length to detector), 57 cm (total length), 50 um I.D. column, and BGE (background electrolyte) of 0.1 M H_3PO_4 brought to pH with NaOH; 0.025 M hydroxypropylbetacyclodextrin; 5% acetonitrile, 30 kV, 200 nm detection. HPLC analyses were carried out on a Varian 5000 HPLC system using a Zorbax SB^{TM} C18 5 um 4.6 mm × 250 mm column,

220 nm, 1 ml/min and radioactivity was monitored with a Ramona Radioactivity Detector with TruCount^{TM} scintillation cocktail in a flow cell. Potassium [^{14}C]cyanide was purchased from IICH through Moravek Biochemicals.

1,6-Hexanedial. To a 100 ml, 2-neck round bottom flask fitted with a thermometer and magnetic stirrer, was added 30 ml each of water and diethyl ether. To this was added cyclohexene (822 mg, 10 mmol) and 2 ml of a 4% aqueous solution of osmium tetroxide (80 mg, 0.3 mmol). To the vigorously stirred dark brown mixture was added sodium periodate (5.25 g, 25 mmol) portionwise at ambient temperature over a period of 45 min. Occasional cooling with an ice/water bath was necessary to maintain the internal temperature at about 25°C. After stirring for additional 3 h, the ensuing white precipitate (sodium iodate) was filtered off and the layers separated. The organic phase was concentrated to dryness *in vacuo* at ambient temperature and the resulting dark brown oil used directly in the next step.

*(S)-2,7-Bis[(R)-2-hydroxy-1-phenylethylamino]-subero[1,8-$^{14}C_2$]nitrile (**2**)*. To the chilled, dark brown oily 1,6-hexanedial (703 mg, 6.2 mmol) in a single neck 100 ml round bottom flask was added sequentially: 30 ml of a chilled aqueous solution of sodium bisulfite (1.28 g, 12.3 mmol), solid potassium [^{14}C]cyanide (638.6 mg, 9.8 mmol, 500 mCi, SA 51 mCi/mmol), and 10 ml of a chilled methanolic solution of (*R*)-(-)-2-phenylglycinol (1.69 g, 12.3 mmol). A magnetic stirring bar was introduced, the flask stoppered, and its contents stirred at ambient temperature for 68 h. The orange colored oily suspension was extracted with ethyl acetate (3 × 40 ml). The aqueous phase (pH 9.9) was diluted to 100 ml with water. The aqueous phase contained 138 mCi, presumably inorganic, and was discarded.

The ethyl acetate extract (362 mCi), containing 66% of the desired diastereomer (**2**) by radio-HPLC (60:40 CH_3CN:0.1% aqueous TFA), was concentrated *in vacuo* to a small volume and placed atop a 20 mm ID × 300 mm Silica (Merck 60, 230–400 mesh) flash chromatography column. The column was eluted with ethyl acetate to give 246 mCi of a diastereomeric mixture free of polar contaminants. This represents a 49% radiochemical yield from potassium [^{14}C]cyanide.

The ethyl acetate eluant (150 ml) was concentrated *in vacuo* to a volume of 15 ml, and 15 × 1 ml injections made onto a 41.4 mm

ID × 250 mm, Rainin Dynamax^{TM}, 60 A, 8 um Silica column, with UV detection (254 nm), 40 ml/min flow rate, using a solvent system of 90:10 ethyl acetate/hexane. The desired diastereomer eluting at 7 min was collected and evaporated *in vacuo* to give 524 mg (146 mCi) of **2** for a 29% radiochemical yield from potassium [^{14}C]cyanide. Compound **2** was a single component by chiral GC assay with a retention time of 16 min. A 73 mCi portion of **2** was used directly in the next step. The structure of the non-isotopic form of **2** was supported by 360 MHz ^{1}H NMR ($CHCl_3$): δH 1.46 (*m*, 4H), 1.74 (*m* 4H), 2.17 (broad s, 4H, OH, NH), 3.23 (*t*, 2H), 3.55 (*m*, 2H), 3.73 (*m*, 2H), 4.08 (*m*, 2H), 7.53 (*m*, 10H).

*(S,S)-2,7-Bis[N-benzylideneamino]subero[1,8-$^{14}C_2$]-nitrile (**5**)*. To a 100 ml round bottom flask fitted with an addition funnel, magnetic stirrer, thermometer and argon inlet, was added methanol (20 ml) and methylene chloride (10 ml). The flask was purged with argon then placed in a dry ice/acetone cooling bath and cooled to an internal temperature of 10°C. Lead tetraacetate (851 mg, 1.9 mmol) was weighed out under argon and then added to the flask in one portion producing a clear, bright, lemon colored solution. The solution temperature was lowered to 0°C and **2** (262 mg, 0.64 mmol, 73 mCi) in methylene chloride (25 ml) added by drops over a period of 44 min. The color of the reaction remained unchanged during the addition. After stirring an additional 30 min at 0°C, saturated aqueous solution of sodium bicarbonate (15 ml) was added. The rapid addition produced a carbon dioxide discharge and a color change to orange accompanied by a temperature increase to 10°C. The mixture was transferred to a 250 ml separatory funnel and the layers separated. The aqueous layer was extracted with methylene chloride (3 × 25 ml). The methylene chloride extracts were combined, washed with saturated aqueous sodium chloride, and then dried over sodium sulfate. The drying agent was removed by filtration and the filtrate evaporated to an oil *in vacuo*. The oil weighed 188 mg (63 mCi) for a radiochemical yield of 86%, and was used directly in the next step.

*(S,S)-2,7-Diamino[1,8-$^{14}C_2$]suberic acid (**6**)*. To **5** (188 mg, 0.55 mmol, 63 mCi) in a 25 ml single neck round bottom flask fitted with a magnetic stirrer was added chilled 6N HCl (9 ml). The mixture was stirred at ambient temperature for 22 h. The benzaldehyde by-product formed an oily suspension, which was removed by extraction with ethyl acetate.

The resulting clear aqueous phase contained 100% of the radioactivity at $R_f = 0.65$ by radio-TLC.

The above aqueous solution was concentrated *in vacuo* (35°C/30 mm Hg) and the resulting residue heated with 12N HCl (10 ml) at 90–95°C for 18 h. A radio-TLC trace showed 95% of the radioactivity was now at $R_f = 0.13$. The aqueous solution was evaporated to dryness *in vacuo* (35°C/30 mm Hg) and the residue treated with 15 N ammonium hydroxide, then lyophilized to dryness. The resulting solid was suspended in absolute ethanol, collected by filtration and then dried *in vacuo* for 21 h at 46°C/30 mm Hg.

The solid weighed 107 mg (56 mCi) for a radiochemical yield of 89%, and a specific activity of 113 mCi/mmol by MS. The material was used directly in the next step.

Mass spectrum (CI, ammonia, *m/z* (%): $(M+H)^+$ 0-^{14}C 205 (3), $(M+H)^+$ 1-^{14}C 207 (28), $(M+H)^+$ 2-^{14}C 209(100).

*(S,S)-2,7-di-boc-diamino[1,8-$^{14}C_2$]suberic acid (**1**).* To a 25 ml single neck round bottom flask fitted with a pH probe, magnetic stirrer and an addition funnel was added **6** (107 mg, 0.5 mmol, 56 mCi) dissolved in 0.2 M aqueous sodium carbonate (5 ml) and acetonitrile (10 ml). The reaction mixture (a thin suspension) was cooled in an ice/water bath and 2.5 N aqueous sodium hydroxide added to pH = 11, forming a solution. A solution of di-tert-butyldicarbonate (352 mg, 1.6 mmol) in acetonitrile (1 ml) was added in one portion. The reaction mixture was stirred in an unreplenished ice/water cooling bath for 3 h, then at ambient temperature for 14 h. A pH = 9 was maintained during the course of the reaction by the occasional addition of 2.5 N aqueous sodium hydroxide. A radio-TLC assay showed 4 spots migrating at R_f 0.16 (2.8%, **6**), 0.55 (90%, **1**) and two unknowns at 0.82 (5.2%) and 0.99 (1.5%).

The reaction mixture was concentrated *in vacuo* (to remove acetonitrile), and diluted with water (40 ml). The pH was 10.4. The aqueous solution was acidified to pH = 1.7 with 1 M aqueous potassium bisulfate. The resulting liberated oil was extracted with ethyl acetate. The extracts were combined, washed with saturated aqueous sodium chloride solution, dried briefly over sodium sulfate, filtered, and the filtrate evaporated to an oil *in vacuo* at 35°C. The oil was crystallized by trituration with acetonitrile. The solid was collected by filtration and dried for 41 h at ambient temperature under high vacuum. The product weighed 120 mg (30 mCi) for a radiochemical yield of 54%. The

radiochemical purity by radio-TLC was 95% ($R_f = 0.68$), specific activity (MS) 113 mCi/mmol with a 95.5% e.e. (*S*, *S*) enantiomeric purity by chiral GC.

Mass Spectrum (CI, Ammonia, m/z (%): $(M+H)^+$ 0-^{14}C, 405 (nil), $(M+H)^+$ 1-^{14}C, 407 (13), $(M+H)^+$ 2-^{14}C, 409 (51).

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References

1. Sakakibara S. *Peptides Prodceedings of the Fifth American Peptide Symposium*; Goodman Meienhofer (ed.). Wiley: New York, 1977; 436.
2. Hiebl J, Blanka M, Guttman A, Kollmann H, Leitner K, Mayrhofer G, Rovenszky F, Winkler K. *Tetrahedron* 1998; **54**: 2059 and references cited therein.
3. Williams RM, Liu J. *J Org Chem* 1998; **63**: 2132 and references cited therein.
4. DeMarsh PL, Wells GI, Lewandowski TF, Bhatnagar PK, Ostivic EJ. *J Infect Dis* 1996; **173**: 205.
5. DeMarsh PL, Sucoloski SK, Frey CL, Koltin Y, Actor P, Bhatnagar PK, Petteway R. *Immunopharmacol* 1994; **27**: 199.
6. Harada K. *Nature* 1963; **200**: 1201.
7. Inaba T, Kozono I, Fujita M, Ogura K. *Bull Chem Soc Jpn* 1992; **65**: 2359.
8. Inaba T, Fujita M, Ogura K. *J Org Chem* 1991; **56**: 1274.
9. Pappo R, Allen DS, Lemieux RU, Johnson WS. *J Org Chem* 1956; **21**: 478.
10. ElAmin B, Anantharamaiah GM, Royer GP, Means GE. *J Org Chem* 1979; **44**: 3442.
11. Mokhallalati M, Pridgen LN. *Synthetic Commun* 1993; **23**: 2055.
12. Hanus J, Veres K. 1970; *J Labelled Compd Radiopharm* 1970; **6**: 143.

