Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **XLI**, 639-646 (1998)

SYNTHESIS OF *cis*-4,5-DIHYDROXY-[3,6-^{14}C]-1,2-DITHIANE-1,1-DIOXIDE

Ken S. Rehder, Maria K. Hristova-Kazmierski and John A. Kepler*

Organic and Medicinal Chemistry, Research Triangle Institute,
Research Triangle Park, NC 27709-2194, USA

SUMMARY

cis-4,5-Dihydroxy-[3,6-^{14}C]-1,2-dithiane-1,1-dioxide ([^{14}C]-**12**) was synthesized in eleven steps from [1,4-^{14}C]fumaric acid ([^{14}C]-**1**) in 10% overall yield.

Key Words: disulfide, anti-HIV, nucleocapsid, CCHC zinc finger, [^{14}C]fumaric acid

INTRODUCTION

The development of new drugs for treatment of HIV has continued to receive considerable attention, partially due to mutational emergence of resistance to compounds currently in clinical use. This problem might be avoided by targeting a highly conserved structure in the retroviral life cycle, thereby circumventing development of resistance. The nucleocapsid protein NCp7, present in most retroviruses (including HIV), contains two highly conserved Cys-X_2-Cys-X_4-His-X_4-Cys sequences.[1] These sequences are known as CCHC zinc fingers, because the cysteines and histidines bind stoichiometrically and with high affinity to a zinc(II) ion.[2] CCHC zinc fingers are necessary for numerous events in viral replication.[3a,b] Due to their conserved nature and their critical role in HIV replication, zinc fingers have been suggested as a logical target for novel anti-HIV drugs.[4]

The hypothesis that HIV NCp7 CCHC zinc finger-targeting compounds might result in HIV-inhibition has recently been realized.[5] The sulfur atoms in the cysteine residues are susceptible to attack by certain functional groups, including disulfides.[6] The resultant covalent modification of the sulfur atoms ejects the bound zinc(II) ion and abolishes

CCC 0362–4803/98/070639–08$17.50

Received 15 December 1997
Accepted 18 February 1998

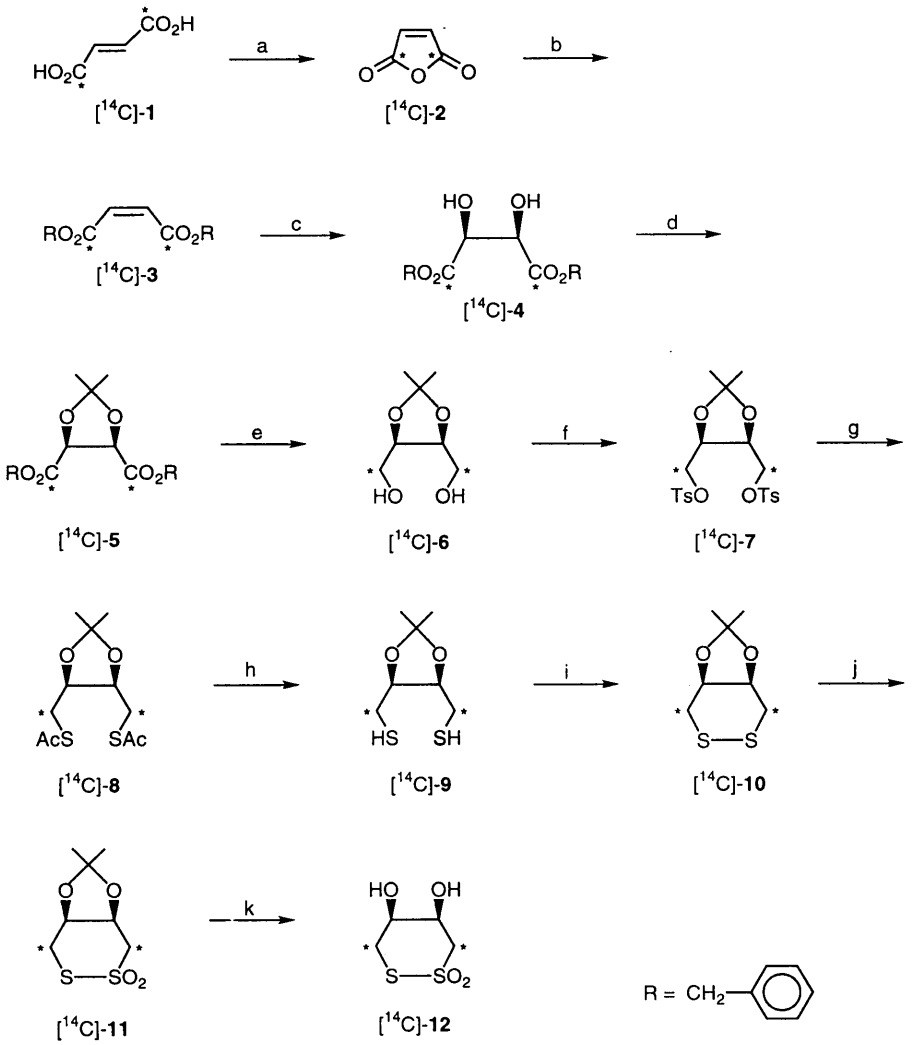
retroviral infectivity. Importantly, there are no viral mutants with immunity to this type of zinc finger-directed chemotherapy. These findings prompted the National Cancer Institute to search their chemical repository for compounds possessing both inhibition of HIV replication and electrophilic functionality. As a result of these screens, NCI has determined that *cis*-4,5-dihydroxy-1,2-dithiane-1,1-dioxide (**12**)[7], possesses CCHC zinc finger-targeting anti-HIV properties.[8] Radiolabeled **12** would aid its pharmacological evaluation. Herein is described the synthesis of carbon-14 labeled **12**.

RESULTS AND DISCUSSION

Synthesis of [^{14}C]-**12** (Scheme 1) was based in part on a sequence of reactions originally described by Carmack and Kelly[9] for the synthesis of *trans*-4,5-dihydroxy-1,2-dithiane. Dehydrative cycloisomerization of [1,4-^{14}C]fumaric acid ([^{14}C]-**1**) with phosphorus pentoxide gave [1,4-^{14}C]maleic anhydride ([^{14}C]-**2**) in 98% yield after vacuum distillation.[10] Esterification of [^{14}C]-**2** with benzyl alcohol and catalytic *p*-toluenesulfonic acid (*p*-TsOH) in refluxing benzene afforded dibenzyl [1,4-^{14}C]maleate ([^{14}C]-**3**) in 95% yield after column chromatography. Stoichiometric dihydroxylation of [^{14}C]-**3** with osmium tetroxide in pyridine provided dibenzyl-*meso*-[1,4-^{14}C]tartrate ([^{14}C]-**4**) in 89% yield.[11] Simultaneous protection of both hydroxyls was achieved by reaction of [^{14}C]-**4** with 2,2-dimethoxypropane and catalytic *p*-TsOH in refluxing benzene to yield acetonide [^{14}C]-**5** in 95% yield.

Synthesis of diol [^{14}C]-**6** by LAH reduction[12] in refluxing Et_2O was straightforward, but isolation of the desired product from the benzyl alcohol by-product required special measures due to the water solubility of [^{14}C]-**6** and its tendency to deketalize during column chromatography on silica gel (see Experimental). Reaction of crude [^{14}C]-**6** with *p*-toluenesulfonyl chloride (*p*-TsCl) in pyridine gave ditosylate [^{14}C]-**7** in 86% overall yield from [^{14}C]-**5** after recrystallization from water. Reaction of [^{14}C]-**7** with potassium thioacetate in refluxing 2-butanone gave bisthioacetate [^{14}C]-**8**, which after filtration to remove precipitated salts was immediately deacetylated with sodium methoxide in methanol to afford intermediate dithiol [^{14}C]-**9**. Air oxidation of [^{14}C]-**9** provided dithiane [^{14}C]-**10**. Oxidation of [^{14}C]-**10** with meta-chloroperoxybenzoic acid (MCPBA) yielded

Scheme 1



dioxide [^{14}C]-**11**, which was then deketalized with 10% aqueous hydrochloric acid-methanol (1:1) to give crude [^{14}C]-**12**. Purification of [^{14}C]-**12** by column chromatography (silica gel, CH_2Cl_2-MeOH, 10:1) gave material which appeared pure by TLC and HPLC; however, examination of the ^{1}H NMR spectrum revealed the presence of an impurity. Use of an alternative eluant for column chromatography (silica gel, Et_2O-MeOH, 20:1) was successful in removing the unidentified impurity, as verified by the ^{1}H NMR spectrum of the product. A total of 9.4 mCi (83.3 mg; 15% chemical yield from [^{14}C]-**7**, 10% radiochemical yield from [^{14}C]-**1**) of [^{14}C]-**12** was obtained which had a specific activity of 20.6 mCi/mmol.

EXPERIMENTAL[13]

Reactions were carried out under a nitrogen atmosphere at ambient temperature unless otherwise indicated. Solutions were dried over sodium sulfate and concentrated on a rotary evaporator under aspirator vacuum. Reagent grade chemicals were purchased from commercial suppliers and used without further purification. Column chromatographies were performed using E. Merck silica gel 60 (230-400 mesh). Analytical TLC was performed using E. Merck silica gel 60 F254 (0.25 mm thickness) plates, and visualized under UV light and/or by staining with 5% phosphomolybdic acid and heat. Radioactive samples were counted using a Packard Tri-carb 4000 liquid scintillation spectrometer in Packard Ultima Gold cocktail using the external standard channels ratio as method of quench correction. TLC-RAM was performed by scanning developed TLC plates using a Bioscan System 200 Imaging Scanner. HPLC was performed using a Rainin HPX dual pump system with a Rheodyne Model 7125 syringe loading injector and IN/US Systems Model 2075 β RAM flow-through radioactivity monitor. ^{1}H NMR were performed using a Brucker WM-250 spectrometer.

[1,4-^{14}C]Maleic anhydride ([^{14}C]-2)[10]

[1,4-^{14}C]Fumaric acid ([^{14}C]-**1**, 0.26 g, 2.2 mmol) and fumaric acid (**1**, 0.27 g, 2.3 mmol) were placed between two layers of P_2O_5 (2.5 g each). The mixture was placed under vacuum (0.1 Torr) and heated to 155-160 °C for 3 h while distilling the product into a liquid N_2-cooled receiver. [^{14}C]Maleic anhydride ([^{14}C]-**2**, 0.43 g, 98% chemical yield) was obtained, suitable for use in the next reaction.

Dibenzyl [1,4-^{14}C]maleate ([^{14}C]-3)

A solution of [^{14}C]-**2** (0.43 g, 4.4 mmol) in anhydrous C_6H_6 (7 mL) was treated with anhydrous benzyl alcohol (1.6 mL, 15.3 mmol) and *p*-TsOH•H_2O (0.20 g, 1.1 mmol). The reaction vessel was equipped with a Dean-Stark trap and the reaction refluxed 24 h. The reaction mixture was cooled and washed with H_2O (1 x 10 mL). The water layer was extracted with Et_2O (3 x 15 mL). The organic layers were combined, washed with saturated aq. $NaHCO_3$ solution (3 x 15 mL) and H_2O (2 x 15 mL) and dried over Na_2SO_4. The solvent was removed under vacuum and the residue was flash chromatographed (SiO_2: hexane-EtOAc, 4:1) to give [^{14}C]-**3** (1.24 g, 95% chemical yield), suitable for use in the next reaction.

Dibenzyl *meso*-[1,4-^{14}C]tartrate ([^{14}C]-4)[11]

A solution of [^{14}C]-**3** (1.24 g, 4.2 mmol) in anhydrous pyridine (15 mL) was treated with OsO_4 (1.0 g, 4.2 mmol). The reaction was stirred 1 h, then treated with a solution of $Na_2S_2O_5$ (1.6 g) in H_2O (30 mL), and pyridine (20 mL). The reaction was stirred 30 min, then extracted with $CHCl_3$ (1 x 150, 3 x 80 mL). The organic extracts were dried over Na_2SO_4, then removed under vacuum to yield [^{14}C]-**4** (1.23 g, 89% chemical yield), suitable for use in the next reaction.

Dibenzyl-2,3-*O*-isopropylidene-*meso*-[1,4-^{14}C]tartrate ([^{14}C]-5)[9]

A solution of [^{14}C]-**4** (1.23 g, 3.7 mmol) in anhydrous C_6H_6 (35 mL) was treated with *p*-TsOH•H_2O (0.03 g, catalytic) and 2,2-dimethoxypropane (0.65 mL, 5.2 mmol). The reaction was heated to reflux for 1.5 h while the C_6H_6-MeOH azeotrope (bp 58 °C) was removed. The reaction was cooled, diluted with C_6H_6 (15 mL), and washed with saturated aq. $NaHCO_3$ solution (3 x 15 mL) and H_2O (1 x 15 mL). The organic extracts were dried over Na_2SO_4, and the solvent removed under vacuum to yield [^{14}C]-**5** (1.30 g, 95% chemical yield), suitable for use in the next reaction.

2,3-*O*-Isopropyliden[1,4-^{14}C]erythritol ([^{14}C]-6)[9,12]

A solution of [^{14}C]-**5** (1.30 g, 3.5 mmol) in anhydrous Et_2O (30 mL) at 0 °C was treated over 5 min with LAH (1.0 M in Et_2O; 5.5 mL, 5.5 mmol). The reaction was warmed to room temperature over 5 min, then heated to reflux for 3 h. The reaction was cooled to 0 °C and treated sequentially with H_2O (210 μL), 15% NaOH (210 μL), and H_2O (630 μL). The resultant heterogeneous mixture was filtered and washed with

Et_2O (20 mL). The ethereal filtrate was dried under vacuum, then azeotroped twice with H_2O (40 mL) under water aspirator vacuum at 50 °C to remove benzyl alcohol. The filtered solids were placed in a micro-Soxhlet extraction apparatus and extracted with refluxing THF (20 mL) for 16 h. The THF extracts were dried under vacuum, and combined with the twice azeotroped ethereal filtrate. The combined material was azeotroped with H_2O (40 mL) under water aspirator vacuum at 50 °C to remove benzyl alcohol, which yielded [^{14}C]-**6**, used directly in the next reaction.

1,4-Ditosyl-2,3-*O*-isopropyliden[1,4-^{14}C]erythritol ([^{14}C]-7)

A solution of [^{14}C]-**6** in anhydrous pyridine (4.5 mL) was treated with *p*-TsCl (1.80 g, 9.4 mmol). The reaction was stirred 19 h, diluted with H_2O and filtered. The solids were washed with H_2O and dried under vacuum to yield [^{14}C]-**7** (1.4 g, 86% chemical yield from [^{14}C]-**5**), suitable for use in the next reaction.

***S,S*-Diacetyl-2,3-*O*-isopropylidene-1,4-dithio[1,4-^{14}C]erythritol ([^{14}C]-8)**

A solution of [^{14}C]-**7** (1.40 g, 3.00 mmol) in 2-butanone (30.0 mL) was treated with KSAc (0.75 g, 6.4 mmol) and heated to reflux for 24 h. The reaction was cooled, filtered and the solvent removed under vacuum to yield [^{14}C]-**8**, used directly in the next reaction.

***cis*-4,5-Isopropylidenedioxy-[3,6-^{14}C]-1,2-dithiane ([^{14}C]-10)**

A solution of [^{14}C]-**8** in anhydrous MeOH (20.0 mL) was treated with NaOMe (0.5 M in MeOH; 1.0 mL, 0.5 mmol) and heated to reflux while the MeOH-MeOAc azeotrope (bp 54 °C) was removed. After ~5 mL of distillate was removed, additional NaOMe (0.5 M in MeOH; 1.0 mL, 0.5 mmol) was added and the process repeated. The solution was diluted with acetone (10 mL), stirred vigorously for 30 min while exposed to atmospheric oxygen, then briefly chromatographed through a plug of silica gel. The solvents were removed under vacuum to yield [^{14}C]-**10**, used directly in the next reaction.

***cis*-4,5-Isopropylidenedioxy-[3,6-^{14}C]-1,2-dithiane-1,1-dioxide ([^{14}C]-11)**

A solution of [^{14}C]-**10** in CH_2Cl_2 (25.0 mL) at 0 °C was treated with MCPBA (0.25 M in CH_2Cl_2; 30.0 mL, 7.5 mmol). The reaction was stirred 5 min and allowed to warm to room temperature over 1 h. The solvent was removed under vacuum to yield [^{14}C]-**11** which was used directly in the next reaction.

cis-4,5-Dihydroxy-[3,6-^{14}C]-1,2-dithiane-1,1-dioxide ([^{14}C]-12)[7]

A solution of [^{14}C]-**11** in MeOH (40.0 mL) was treated with 10% aq HCl (40.0 mL) and stirred 16 h. The solution was concentrated, filtered, and the solvent removed under vacuum to yield crude [^{14}C]-**12**. This material was flash chromatographed (SiO_2: CH_2Cl_2-MeOH, 10:1) to give material (0.14 g) which appeared pure by TLC-RAM (5 x 20 cm silica gel plate: CH_2Cl_2-MeOH, 10:1) and HPLC-RAM (Beckman Ultrasphere ODS 4.6 x250 mm column; 0.01 M phosphate buffer, pH 5 mobile phase; 1.0 mL/min flow rate). Examination of the ^{1}H NMR spectrum (d_6-acetone; 250 MHz), however, revealed the presence of an impurity. Removal of the impurity was achieved by flash chromatography (SiO_2: Et_2O-MeOH, 20:1). Analysis of the product by HPLC (Zorbax Rx-C8 4.6 x 250 mm column; H_2O-CH_3CN , 40:1; 1.0 mL/min flow rate) and ^{1}H NMR (d_6-acetone solvent; 250 MHz) confirmed the removal of the impurity.

A total of 83.8 mg (15% chemical yield from [^{14}C]-**7**) of [^{14}C]-**12** was obtained which was 99% radiochemically pure by TLC-RAM (SiO_2: Et_2O-MeOH, 20:1) and 98% radiochemically pure by HPLC-RAM (second system above). The mp (134 °C, lit.[7] 134-138 °C) and the ^{1}H NMR spectrum indicated the chemical purity was commensurate with the radiochemical purity. The specific activity was determined[14] to be 112 μCi/mg (20.6 mCi/mmol) and the total radioactivity was 9.4 mCi.

ACKNOWLEDGMENT

The authors would like to thank Mr. George F. Taylor for invaluable technical assistance, and Ms. Ann Allen for preparation of this manuscript. This work was supported under Contract No. No1-CM-47008 with the National Cancer Institute.

REFERENCES

1. Berg J. M. and Shi Y. – Science 271: 1081 (1996).
2. South T. L, Blake P. R., Sowder, R. C. II, Arthur L. O., Henderson L. O., Summers L. F. – Biochemistry 29: 7786 (1990).
3. (a) Turpin J. A., Schaeffer C. A., Terpening S. J., Graham I., Bu M., Rice W. G. – Antiviral Chem. Chemother. 8: 60 (1997). (b) Turpin J. A., Terpening S. J., Schaeffer C. A., Yu G., Glover C. J., Felsted R. L., Sausville E. A., Rice W. G. – J. Virology 70: 6180 (1996).

4. Rice W. G., Supko, J. G., Malspis L., Buckheit R. W. Jr., Clanton D., Bu M., Graham L., Schaeffer C. A., Turpin J. A., Domagala J., Gogliotti R., Bader J. P., Halliday S. M., Coren L., Sowder R. C. II, Arthur L. O., Henderson L. E. – Science 270: 1194 (1995).
5. McDonnell N. B., DeGuzman R. N., Rice W. G., Turpin J. A., Summers M. F. – J. Med. Chem. 40: 1969 (1997) and references contained therein.
6. Hathout Y., Fabris D., Han M. S., Sowder R. C. II, Henderson L. E., Fenselau C. – Drug Metab. Dispos. 24: 1395 (1996).
7. Singh P. K., Nguyuen T. Q., Field L. – Sulfur Letters 8: 107 (1988).
8. Rice W. G., Baker D. C., Schaeffer C. A., Graham L., Bu M., Terpening S., Clanton D., Schultz R., Bader J. P., Buckheit R. W. Jr., Field L., Singh P. K., Turpin J. A. – Antimicrob. Agents Chemother. 41: 419 (1997).
9. Carmack M. and Kelley C. – J. Org. Chem. 33: 2171 (1968).
10. Nystrom R. F., Loo Y. H., Leak J. C. – J. Am. Chem. 74: 3434 (1952).
11. Baran J. S. – J. Org. Chem. 25: 257 (1960).
12. Feit P. W. – J. Med. Chem. 7: 14 (1964).
13. All compounds gave spectral and physical data consistent with the proposed structures.
14. The specific activity is the average of three separate determinations by weighing and liquid scintillation counting.