Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **42**, 827-834 (1999)

SYNTHESIS OF CARBON-14 LABELED CI-1012 AND CI-1013, POTENTIAL ANTI-HIV AGENTS.

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SUMMARY

^{14}C -Labeled CI-1012 and CI-1013 of the 2,2'-dithiobis[benzamide] and benzisothiazolone series, inhibitors of viral replication and potential anti-HIV agents, were prepared. The radiolabeling of CI-1013, a six-step synthetic sequence, started with the treatment of *N-t*-BOC-aniline (**2**) with 2.0 equivalents of *t*-BuLi, followed by carboxylation of the *ortho*-lithiated center in the resulting lithiated dianion **2b** with [^{14}C]carbon dioxide, to give *N-t*-BOC-2-Amino-[7-^{14}C]benzoic acid (**3**). Product **3** was then deprotected with trifluoroacetic acid to give 2-Amino-[7-^{14}C]benzoic acid as its TFA salt (**4**). The latter was diazotized and treated with Na_2S_2, generated in situ, to give 2,2'-dithiobis[7-^{14}C]benzoic acid (**5**). The acid chloride from **5**, obtained by treatment with thionyl chloride, was subsequently coupled with L-isoleucine *tert*-butyl ester and deprotected with TFA to produce [*S*-(*R**,*R**)]-2-{2-[2-(1-Carboxy-2-methylbuty)[^{14}Clcarb-amoyl)phenyldisulfanyl]-[7-^{14}C]benzoylamino}-3-methylpentanoic acid ([$^{14}C_2$]CI-1013) (**8**). Treatment of **8** with bromine yielded the benzisothiazolone, [*S*-(*R**,*R**)]- 3-Methyl-2-(3-oxo-3*H*-[3-^{14}C]benz[*d*]isothiazol-2-yl)pentanoic acid ([^{14}C]CI-1012) (**9**).

Keywords: Carbon-14 synthesis, 2-amino-[7-^{14}C]benzoic acid, 2,2'-dithiobis[7-^{14}C]benzoic acid, CI-1013, CI-1012, benzisothiazolone, 2,2"-dithiobis[benzamide], anti-viral (HIV) agents.

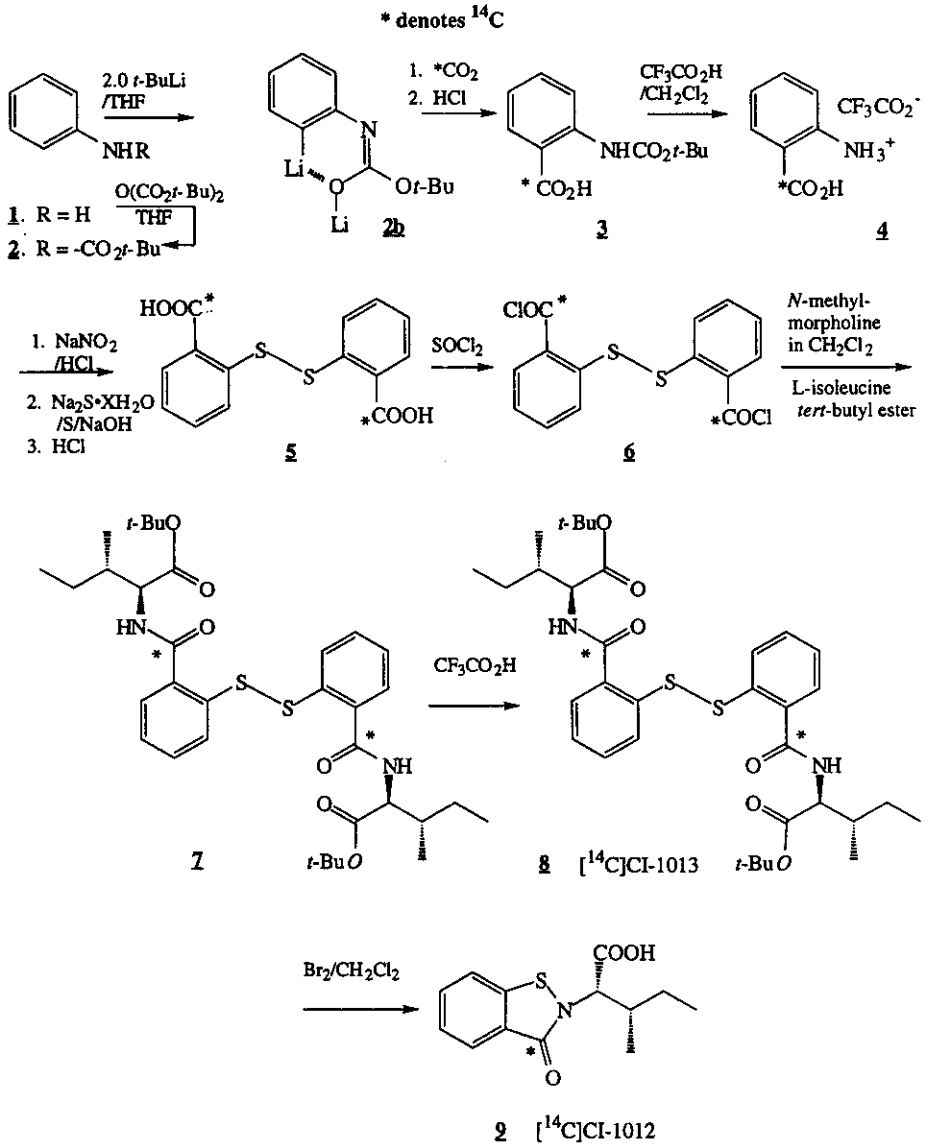
INTRODUCTION

The spread of the human immunodeficiency virus (HIV) is continuing with an alarming estimate of 30 million indi viduals infected by the year 2000. Conceptually the HIV-1 life cycle offers many possible sites of chemotherapeutic intervention, relating to reverse transcriptase, integrase,

CCC 0362–4803/99/090827–08$17.50

Received 18 September 1998
Revised 26 January 1999
Accepted 29 January 1999

Scheme 1. Synthesis Of Carbon-14 Labeled CI-1012 and CI-1013.



protease, nucleocapsid protein, RNA-polymerase, and other accessory proteins. Nevertheless presently available drugs consist mainly of multiple nucleotides targeted to reverse transcriptase and several HIV-1 protease inhibitors.[1,2]

The monumental rate of HIV viral replication is accompanied by rapid resistance development to single and even multiple agents. Thus combination therapy, especially with novel agents and/or

novel targets not cross resistant with existing therapeutic strategies, has become necessary. Also, from economic consideration, the increase in number of drugs required point to the importance of readily-synthesized novel agents with relatively uncomplicated structures.[1,2]

CI-and 1013 represent members of the of 2,2'-dithiobis[benzamide] and benzisothiazolone series, which have been shown to inhibit HIV viral replication in vitro and are being developed as a new class of potential anti-HIV agents. HIV nucleocapsid protein (NCp7) has been suggested as a possible target, and biophysical characterization of zinc ejection from NCp7 by these compounds and a possible mechanism were reported recently.[1,2,3] The C-14 labeled forms of the compounds were prepared for pharmacokinetic and metabolic studies.

CHEMISTRY

As shown in Scheme 1, [$^{14}C_2$]CI-1013 (**8**) was synthesized in six steps starting from *N-t*-BOC-aniline (**2**). [^{14}C]CI-1012 was then synthesized in one step from **8**.

Thus *N-t*-BOC-aniline (**2**), obtained in 53% yield from reaction of aniline (**1**) with di-*tert*-butyl dicarbonate,[4] was treated with *t*-BuLi to generate the corresponding lithiated dianion (**2b**).[5] In contrast to the reported procedure using 2.4 equivalents (20% excess) of *t*-BuLi, exactly two equivalents was used. The *ortho*-lithiated carbon in the dianion **2b** thus generated was then carboxylated with one equivalent of [^{14}C]carbon dioxide to give the *N-t*-BOC protected 2-amino[7-^{14}C]benzoic acid (**3**). The crude product showed a radiochemical purity of 82 to 83% by thin-layer chromatography (TLC). The estimated radiochemical yield of pure **3** present in the crude product was 56 to 60% in two runs. The use of exactly two equivalent of *t*-BuLi to each equivalent of **2** led to approximately 50% improvement in yield over that from the use of 20% excess, since excess *t*-BuLi would react with the labeling reagent [^{14}C]carbon dioxide. The yield possibly could have been further improved by using an excess of the dianion **2b**, relative to [^{14}C]carbon dioxide, to achieve complete utilization of the latter.[6]

Crude product **3** (estimated to contain 2.4 mmol of **3**, 132 mCi), was diluted approximately one-fold with cold anthranilic acid treated with trifluoroacetic acid (TFA) in dichloromethane to give the TFA salt of 2-amino-[7-^{14}C]benzoic acid ([7-^{14}C]anthranilic acid) (**3**), obtained as solid upon evaporation. The product was diazotized and treated with Na_2S_2,[5] in presence of excess base, to

give crude dithio[7-^{14}C]anthranilic acid (5) in 92% yield. The crude acid 5 was treated with thionyl chloride (8 h, 83-87 °C) to give the dichloride (6) which, upon prolonged drying in high vacuum, suffered some volatile loss, leaving 91% of the theoretical weight of product. The dichloride 6 was treated with L-isoleucine *tert*-butyl ester to give the di-*tert*-butyl ester (7).[3] The crude product was passed through 1.7 times its weight of silica gel to remove low Rf impurities, followed by crystallization from dichloromethane/ethyl acetate mixtures to give 62% yield of crystalline product 7. The overall radiochemical yield of 7 from 3 was 53%.

The di-*tert*-butyl ester 7, 350 mg, was converted to CI-1013 (8) by treatment with trifluoroacetic acid. The crude product was purified by crystallization from THF/hexanes and ether/hexanes mixtures to give 281 mg of CI-1013 (8), 107.2 μCi/mg, 30.1 mCi (94% radiochemical yield).

[^{14}C]CI-1012 (9) was then prepared from 168 mg of [^{14}C]CI-1013 (8) by treatment with bromine in dichloromethane[3] in almost quantitative yield. The compound was crystallized from *tert*-butyl methyl ether/hexanes mixture to give 138 mg of crystalline product, 107.8 μCi/mg (28.6 mCi/mmol), 14.8 mCi (83% radiochemical yield). From the mother liquor another 21.8 mg (14.6%) of essentially pure product was recovered.

The overall radiochemical yield for [^{14}C]CI-1013 (8) was calculated to be approximately 29%, and that for [^{14}C]CI-1012 (9), 24%, based on [^{14}C]CO_2 used.

EXPERIMENTAL

General. Radiochemical counting was performed with a Packard Tri-Carb 4530 or 2300 TR liquid scintillation analyzer, using Beckman Ready-Gel or Packard Ultima Gold XR LSC-cocktail as the counting medium. TLC was performed with Silica Gel 60 F_{254} precoated plates by EM Science and were scanned on a Berthold LB2832 automatic TLC linear analyzer or a Bioscan System 200 imaging scanner. HPLC analyses of the final products were performed on a Water Associates 600E system with on-line Applied Biosystems 1000S diode array detector and either a β-RAM radioactivity detector or Radiomatic series A-200 radioactivity flow detector. HPLC was performed with Alltech Econosil C18-10μ analytical columns, 4.6 mm x 250 mm, unless otherwise specified. THF (tetrahydrofuran) was purified by distillation over sodium and benzophenone. Drying of non-hydroxylic solvents, if indicated, was generally accomplished with Molecular Sieves. All labeled compounds synthesized were identified by TLC or HPLC comparison, or both, as well as by NMR comparison, with the corresponding authentic unlabeled compounds.

N-t-BOC-2-Amino-[7-^{14}C]benzoic acid (3) (*N-t*-BOC-[7-^{14}C]anthranilic acid). Barium [^{14}C]carbonate (5.09 mmol, 1013 mg, 283 mCi) was treated with 25 mL of concentrated H_2SO_4 in a flask attached to a vacuum-line apparatus (room temperature, then 10 min at 40 to 50 °C near the end of the reaction). The $^{14}CO_2$ generated was stored in a reservoir closed with a stopcock and cooled with liquid nitrogen. A 100-mL 2-necked flask, containing 1.934 g (10.0 mmol) of *N-t*-BOC-aniline (2), was attached to the vacuum-line apparatus, which was then filled with argon, and 40 mL of THF was introduced. The solution was cooled at -78 °C, and 1.5 N *t*-BuLi in hexane was added dropwise with stirring until a permanent yellow tinge was obtained (1.0 equivalent, 7.0 mL, 4.412 g, in about 1h); exactly one more equivalent (4.430 g) was then added in 15 min. The bath temperature was allowed to warm to -32 °C in 70 min, then maintained at -22 °C (Dry-Ice, CCl_4) for 1.0 h. The solution, containing dianion of 2, was then cooled in a liquid nitrogen bath and evacuated and filled with the $^{14}CO_2$ from the reservoir, transferred by sublimation under static vacuum. The bath was replaced with a Dry-Ice acetone bath, and the reaction mixture was stirred vigorously while the bath was allowed to warm up slowly to room temperature overnight.

The deep red reaction mixture was combined with 75 mL of ether and extracted with 5% $NaHCO_3$ (4x30 mL, 1x10 mL). The aqueous extract was washed with ether (2x20 mL), and the ether wash was individually extracted with $NaHCO_3$ (10 mL). The combined aqueous extracts were acidified with 41 g of citric acid added in portions, and the gas evolved was bubbled through 4 N NaOH to trap any unreacted $^{14}CO_2$. The acidified solution was extracted five times with CH_2Cl_2 (total of 180 mL), and the extract was dried (6 g Na_2SO_4) and evaporated to give 883 mg (73% yield) of crystalline solid, 218 μCi/mg (193 mCi total), radiochemical purity of 82% by TLC ($CHCl_3$:THF/82:18; Rf 0.26). The crude product thus contained 158 mCi of 3 (56% radiochemical yield). (By using an excess of the *N-t*-BOC-aniline dianion, complete utilization of the $^{14}CO_2$ probably could have been achieved, thereby increasing the radiochemical yield and avoiding the use of NaOH trap).[6]

In two other similar runs using 1 eq of $^{14}CO_2$, 2.0 eq of *N-t*-BOC-aniline (2), and 2.4 eq of *t*-BuLi (instead of 2.0 eq above), the crude weight yield of 3 was 46 and 51%, respectively (based on $^{14}CO_2$ used, vs. 73% above).

2-Amino[7-^{14}C]benzoic acid trifluoroacetate (4). A solution of the crude product prepared above, containing 132 mCi of 3 by calculation (2.37 mmol based on 55.6 mCi/mmol) in 15 mL of CH_2Cl_2 was combined with 307 mg of unlabelled anthranilic acid (2.24 mmol) in a 100-mL flask, and 5.0 mL of trifluoroacetic acid was added in 15 min with stirring. After 2.5 h, the solution was evaporated in vacuo to 3.1 g of dark liquid, which was dissolved in 21 mL of dichloromethane, and the solution was again evaporated to give a solid, 1.276 g after drying for 20 h over KOH under high vacuum. Based on a total of 4.61 mmol of anthranilic acid, the calculated mol wt is 276 and corresponds to the presence of 1.2 eq of trifluoroacetic acid.

2,2'-Dithiobis[7-^{14}C]benzoic Acid (5). Compound 4, 1.27 g (131 mCi, 4.6 mmol) was diazotized and reacted with Na_2S_2 according to the detailed procedure reported for the synthesis of

unlabeled thiosalicyclic acid,[4] with the exception that an additional 1.3 equivalent of NaOH was added to the Na_2S_2 solution because of the presence of 1.2 equivalent of TFA solvate in the starting material. The product, 653 mg (2.12 mmol; 92%), showed a radio-chemical purity of 91% by TLC ($CHCl_3$:THF:HOAc/52:7.2:0.8, Rf = 0.61) (approx. 121 mCi of 5).

2,2'-Dithiobis[7-^{14}C]benzoyl Chloride (6). A mixture of the crude acid 5 above (651 mg) and $SOCl_2$ (24.3 g) was heated with magnetic stirring at an oil bath (85–89 °C; slight reflux) for 8 h. Upon evaporation in vacuo, crystallization occurred. The product was dried in high vacuum for 17 h to 721 mg (99% yield). Further drying for one day resulted in loss of product by volatilization, giving 662 mg (1.92 mmol) of 6 (91%) (approx. 110 mCi).

[*S*-(*R,*R**)]-2-{2-[2-(1-Carboxy-2-methylbuty[^{14}C]carbamoyl)phenyldisulfanyl]-[7-^{14}C]benzoylamino}-3-methylpentanoic acid di-*tert*-Butyl Ester (7).** To a stirred solution of L-isoleucine *tert*-butyl ester hydrochloride (Bachem 867 mg, 3.96 mmol) in 1.5 mL of CH_2Cl_2 at 0 °C was added slowly a solution of 809 mg of N-methylmorpholine (99%, 7.91 mmol) in 8.5 mL of CH_2Cl_2. The acid chloride 5 (1.92 mmol), dissolved in portions of CH_2Cl_2 (12 mL total), was added in about 15 min. The ice-bath was then removed. After 85 min, TLC (hexanes:CH_2Cl_2:EtOAc /5:4:1; Rf of product, 0.41) showed that the reaction was essentially complete. After an additional 22 h at 0 °C, TLC showed that more impurities had formed with slightly higher Rf's. The reaction mixture was diluted with 6 mL of CH_2Cl_2 and washed with water (2x2.5 mL), 0.25 M citric acid (3.1, then 1.6 mL), and water (2x2.5 mL). Extraction of the aqueous wash fractions with 5 mL of CH_2Cl_2 gave only 0.4 mCi of material. The CH_2Cl_2 extracts were passed through, in sequence, 2.01 g of silica gel to remove a small amount polar impurity (Rf 0). The eluate was evaporated to dryness, dissolved in 3.3 mL (4.3 g) of CH_2Cl_2, and 19 mL (17 g) of EtOAc (50 °C) was added. The solution was evaporated to remove 6 g of solvent and allowed to cool. The crystals which formed was filtered and washed with cold EtOAc to give 484 mg of 7 with minute trace of two higher Rf's impurities. The filtrate was diluted with 3.32 mL (4.4 g) of CH_2Cl_2 and evaporated to about 7 g of solution to provide another 161 mg of essentially pure 7. The column was further eluted with 10 mL of hexane: CH_2Cl_2:EtOAc/5:4:1, the eluate was combined with the various mother liquors, and the solute recovered upon evaporation was recrystallized. All moderately pure fractions thus obtained were combined and chromatographed over a small silica gel column to give, after repeated fractional crystallizations, another 53 mg of 7. The total yield of 7 was 762 mg (1.18 mmol; 62%), 91.5 μCi/mg, 70 mCi (53% overall radiochemical yield from 3).

[*S*-(*R,*R**)]-2-{2-[2-(1-Carboxy-2-methylbuty[^{14}C]carbamoyl)phenyldisulfanyl]-[7-^{14}C]benzoylamino}-3-methylpentanoic acid (CI-1013) (8).** To a solution of 7, 70 mg (0.108 mmol, 6.4 mCi) in 2.0 mL of CH_2Cl_2, was added with swirling 1.75 mL of TFA. After 1 day the solution was evaporated to dryness in vacuo (bath temperature 28 °C) and then subjected to high vacuum for a day. The residual solid was dissolved in 830 mg of THF, and 845 mg of hexanes was added with stirring. Crystallization occurred, and another 140 mg of hexanes was added. To the thick crystalline paste was added 4.35 g of hexane:ether/2:1. The mixture was filtered, washed with

the hexane:ether/2:1 and finally with pentane to give, after drying, 48.4 mg of **8**. The procedure was repeated with 280 mg (0.432 mmol, 25.6 mCi) of **7** to give 236 mg of product. Drying in high vacuum for 55 h resulted in 0.4% of weight loss. The total yield was thus 281 mg (0.526 mmol), 107.2 μCi/mg, 30.1 mCi (94% radiochemical yield). HPLC (Econosil CN 10 μ, hexane:THF:TFA/72:28:0.1, 2.0 mL/min, 251 nm, with sample dissolved in THF:mobile phase/1:2 for injection): chemical purity 98.7% (8.46 min); radiochemical purity 98.5% (8.32 min); major impurity at 5.1 min, 0.75% chemical, 0.83% radiochemical. NMR (Me_2SO-d_6) δ 0.82 (t, 6H), 0.90 (d, 6H), 1.25 (m, 2H), 1.45 (m, 2H), 1.89 (m, 2H), 4.29 (t, 2H), 7.25 (t, 2H), 7.40 (t, 2H), 7.59 (d, 4H), 8.68 (d, 2H), 12.63 (s, 2H); the spectrum was identical to that of an authentic sample.

[*S*-(*R,*R**)]-3-Methyl-2-(3-oxo-3*H*-[3-^{14}C]benz[*d*]isothiazol-2-yl)pentanoic acid (CI-1012) (9).** A mixture of 168 mg (0.314 mmol, 18 mCi) of **8** in 1.7 mL of CH_2Cl_2 was stirred magnetically to give a paste, and 11.1 mL of a CH_2Cl_2 solution of bromine, 0.0388M (50 μL of bromine in 25 mL of solution), was added. The vigorously stirred mixture became clear in 7 min, and crystallization began in about 10 min. After 14.5 h the mixture was evaporated to dryness in vacuo and partitioned between 8.5 mL of CH_2Cl_2 and 3.9 mL of 6.2% $NaHCO_3$. The organic layer was further extracted twice with 6.2% $NaHCO_3$ (3.9 mL, 0.5 mL). The combined aqueous fractions were adjusted to pH 1.27 with 0.677 g of 12 N HCl, added dropwise with stirring. The aqueous phase was extracted with CH_2Cl_2 (10mL, then 2x6 mL). The three CH_2Cl_2 fractions were washed sequentially with a 3.6-mL portion of water, combined, and dried with anhydrous magnesium sulfate (73 mg). TLC (chloroform:THF:HAc/80:18:2), Rf 0.56, radiochemical purity 99.8%. The solution was evaporated and dried in high vacuum to 161 mg of a solid foam. A solution of the solid in *t*-BuOMe was evaporated to 900 mg, crystallization occurred after seeding, and more hexanes was added. The crystals were filtered and washed with *t*-BuOMe: hexanes/1:1.5 and then with *n*-pentane to give, after drying in high vacuum, 138 mg (0.518 mmol) of **9**, 108 μCi/mg (approximately 28.6 mCi/mmol), 14.9 mCi (83% radiochemical yield). HPLC (Hypersil BDS C18 5μ, 4.6x250mm, 0.5% triethylamine adjusted to pH 2.8 with phosphoric acid:THF/65:35, 1 mL/min, UV 230 nm; retention time 7.2 min): radiochemical purity 99.8%; chemical purity 99.5%. NMR (Me_2SO-d_6) δ 0.84 (t, 3H), 1.01 (d, 3H), 1.18 (m, 1H), 1.28 (m, 1H), 2.12 (m, 1H), 5.00 (d, 1H), 7.48 (t, 1H), 7.73 (t, 1H), 7.92 (dd, 2H); the spectrum was identical to that of an authentic sample. From the mother liquor another 21.8 mg (14.6% yield) of essentially pure product was recovered.

ACKNOWLEDGMENTS

The authors express grateful appreciation to Yun Huang and Michael Asuncion for analyses, to Michael Stier and Rocco Gogliotti for helpful assistance, and to Drs. Joseph Sanchez, Jonathan Walker, John Domagala, and Om Goel for insightful information and discussions.

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6. Retrospective consideration of experimental results led to this suggested improvement of procedure, which could further enhance radiochemical yield and mitigate radioactive-waste disposal. However, this suggestion was not tested due to the urgent priority of utilizing the reaction product **3** to obtain the final target compounds **8** and **9**.

