Synthesis of Carbon-14 and Tritium Analogues of the Phospholipid Antitumor Agent SDZ 62-834 zi

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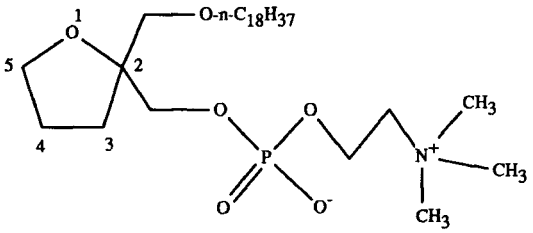
SUMMARY

2-[[Hydroxy[[tetrahydro-2-[(octadecyloxy)methyl]-2-furanyl)]methoxy]phosphinyl]oxy]-*N, N, N*-trimethylethanaminium hydroxide inner salt (SDZ 62-834 zi) was synthesized with carbon-14 at the furan-2 position from dimethyl malonate-[^{14}C]. In addition, SDZ 62-834 zi was also synthesized with tritium at the furan 3 and 4 positions by catalytic tritiation of 2,5-dihydro-2,2-furandimethanol.

Key Words : phospholipid, tetrahydrofuran, carbon-14, tritium, rhodium carbenoid.

Introduction

The tetrahydrofuran phospholipid, SDZ 62-834 zi, **1**, has been shown to exhibit antineoplastic activity *in vivo* against a wide variety of tumor cell lines and is currently in clinical trials[1]. Radiolabeled analogues of SDZ 62-834 zi were therefore required for ADME studies in animals and man. The synthesis of the carbon-14 isotopomer, **2**, featured a rhodium(II)-^{14}C-carbenoid mediated *O-H* insertion reaction. However, due to the radiochemical instability of **2**, the tritium analog **3** was prepared and has demonstrated satisfactory radiochemical stability. In addition, **3** has been shown to be metabolically stable in the rat[2].



1 SDZ 62-834 zi

2 [2-^{14}C] SDZ 62-834 zi

3 [3,4-^{3}H] SDZ 62-834 zi

Discussion

The synthesis of SDZ 62-834 zi-2-^{14}C, **2**, is outlined in Scheme 1. Dimethyl malonate-2-^{14}C, **4**, was treated with mesyl azide[3] and triethylamine to give the diazo compound **5** by a diazotransfer reaction. Dimethyl diazomalonate, **5**, was then heated in the presence of rhodium(II)acetate dimer and

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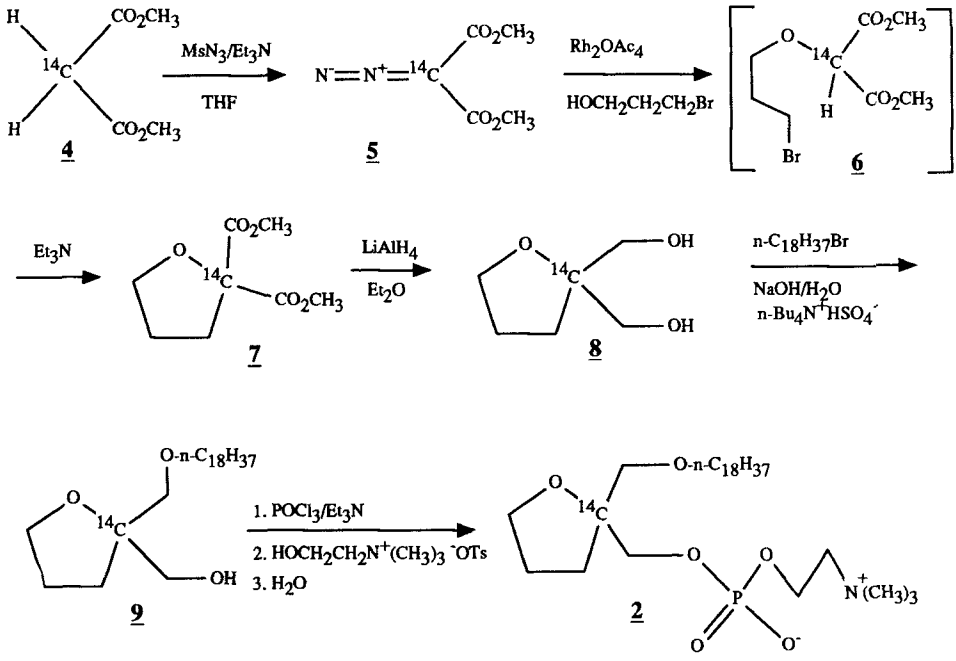
3-bromopropanol to give the ether **6**. The reaction proceeds *via* a bimolecular rhodium carbenoid *O-H* insertion reaction[4]. The fact that the carbon-14 was the reacting site did not appear to alter the normal course of the reaction. The tetrahydrofuran diester, **7**, was formed by treatment of intermediate **6** (not isolated) with triethylamine. The ester groups were then readily reduced with lithium aluminum hydride to give the tetrahydrofuran dimethanol **8**. Monoalkylation of this diol with stearylbromide utilized a two-phase reaction system employing *n*-tetrabutylammonium hydrogen sulfate as phase transfer catalyst to yield **9**. Monoether **9** was then subjected to a three-step, one-pot operation which gave rise to the title material, **2**. Thus, treatment of **9** with phosphorous oxychloride and triethylamine gave an intermediate dichlorophosphate that was further treated with choline tosylate, pyridine and catalytic dimethylaminopyridine (DMAP). Addition of water to the reaction vessel generated crude **2** which was purified first through an ion-exchange column and then by silica gel flash column chromatography[5].

Scheme 1

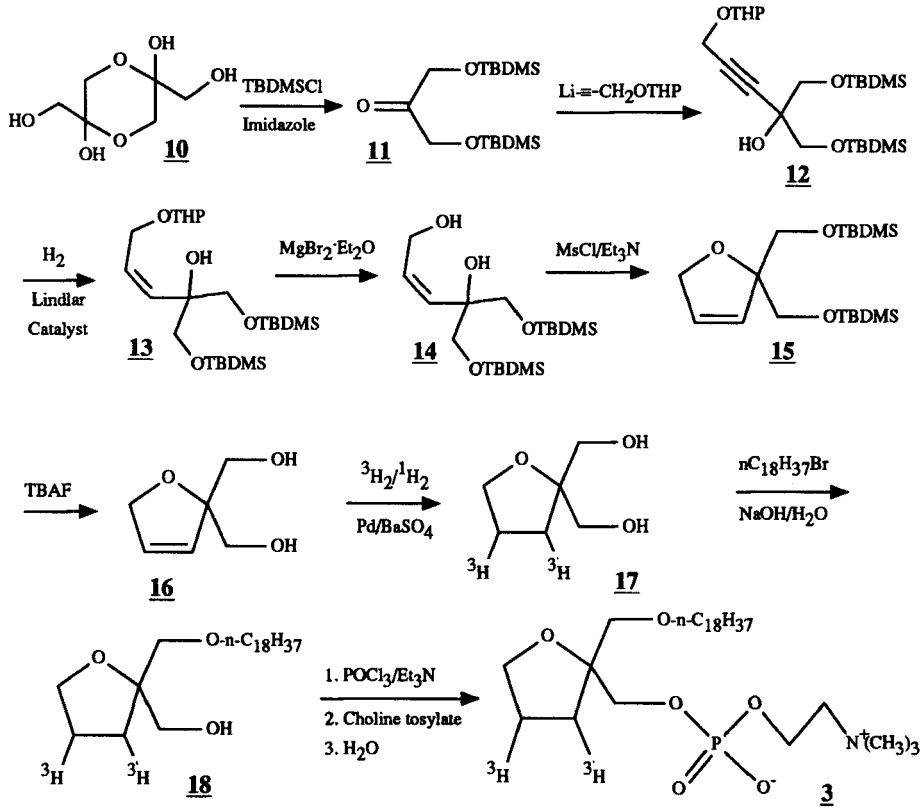


Unfortunately, **2** proved to be radiochemically unstable even at low specific activity (4 μCi/mg). Over a period of six months, the material decomposed by approximately 27%.

Concurrent to the carbon-14 synthesis, an alternative tritium-labeled material was sought. The synthesis of this compound, **3**, is given in Scheme 2. 1,3-Dihydroxyacetone dimer, **10**, was converted to the corresponding disilyl protected diether **11** using *t*-butyldimethylsilyl chloride and imidazole in dimethylformamide (DMF). The tertiary propargylic alcohol **12** was obtained upon reaction of this

diether with the lithium salt of tetrahydropyran (THP) protected propargyl alcohol. Partial hydrogenation of **12** using hydrogen gas and Lindlar's catalyst accessed *cis*-olefin **13**. The THP-group was removed by the action of magnesium bromide etherate[6] giving rise to butenediol **14**. Ring closure of this material was effected by mesyl chloride/triethylamine to give dihydrofuran **15**. Fluoride assisted desilylation of **15** led to dihydrofurandimethanol **16** which was tritiated with $^3H/^1H$ gas in the presence of palladium catalyst to give labeled tetrahydrofuran **17**. This compound was then treated in the same fashion as **8** (Scheme 1) to yield the title compound, [^{3}H] SDZ 62-834 zi, **3**, which has thus far exhibited satisfactory radiochemical stability (at 95 μCi/mg). Both **2** and **3** have been shown to be identical in all aspects to unlabeled drug substance.

Scheme 2



Experimental

Dimethyl malonate-2-[^{14}C] was purchased from Amersham Corp. and American Radiolabeled Chemicals Inc. Tritium gas was obtained from Amersham Corp. The NMR spectra were obtained on JEOL FX 200 and Bruker AC 300 spectrometers. Chemical ionization mass spectra were recorded on a Finnigan MAT 4600 mass spectrometer and used ammonia as the reagent gas. Fast atom bombardment mass spectra were recorded on a VG 7070E mass spectrometer. Ion production was *via*

fast atom bombardment using a xenon primary beam of 6 KeV energy on samples introduced in a thioglycerol matrix.

2-Diazopropanedioic-[2-^{14}C] acid dimethyl ester, 5

To a stirred solution of propanedioic-2-[^{14}C] acid dimethyl ester, 4, (145 mCi, 1.86 g, 14.1 mmol) and methanesulfonyl azide[3] (2.60 g, 21.2 mmol) in 50 mL of tetrahydrofuran (THF) at room temperature was added dropwise triethylamine (4.0 mL, 28.2 mmol). The resultant solution was stirred at 25 °C for 3 days and then concentrated *in vacuo*. The residue was purified by silica gel chromatography (20% ethyl acetate-hexane) to give 130.7 mCi (90 %) of 5 as a yellow oil. CI-MS (MH^+) 159.

Dimethyl tetrahydrofuran-[2-^{14}C]-2,2-dicarboxylate, 7

To a stirred solution of 5 (130.7 mCi, 12.7 mmol) and 3-bromopropane (1.15 mL, 12.7 mmol) in 75 mL of dichloromethane at 25 °C was added in one portion 50 mg of rhodium(II)diacetate dimer. The mixture was stirred at reflux for 18 hours and then triethylamine (3.5 mL, 25.4 mmol) was added in one portion. The resultant solution was stirred an additional 48 hours at room temperature. The reaction mixture was then suction-filtered through a pad of silica gel and the pad was rinsed with 500 mL of diethyl ether. The filtrates were concentrated *in vacuo* to give 111 mCi (85%) of the title compound, 7, as a clear oil. CI-MS (MH^+) 189; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.05 (m, 2H), 2.50 (t, 2H, j=7.5 Hz), 3.82 (s, 6H), 4.10 (t, 2H, j=7.5 Hz).

Tetrahydro-[2-^{14}C]-2,2-furandimethanol, 8

Under a blanket of nitrogen, a solution of 7 (111 mCi, 10.8 mmol) in 15 mL of diethyl ether was added dropwise to a stirred suspension of lithium aluminum hydride (1.4 g, 38.1 mmol) in 40 mL of diethyl ether at 5 °C. The reaction was stirred under these conditions for 2.5 hours and was then quenched by the careful sequential addition of 1.5 mL of water, 1.5 mL of 15% NaOH and 4.5 mL of water. The resultant suspension was diluted with 50 mL of methanol and stirred at room temperature for an additional 16 hours. The mixture was suction-filtered through a pad of silica gel and the pad was washed with 500 mL of methanol. The filtrates were concentrated under reduced pressure to give 61.1 mCi (55%) of tetrahydro-[2-^{14}C]-2,2-furandimethanol, 8, as an opaque gum. CI-MS (MH^+) 133.

Tetrahydro-[2-^{14}C]-2-[(octadecyloxy)methyl]-2-furanmethanol, 9

A solution consisting of 8 (61.1 mCi), octadecyl bromide (4.2 g, 12.70 mmol) and tetrabutylammonium hydrogen sulfate (4.3 g, 12.7 mmol) in 50 mL of toluene was stirred at 60 °C for 10 minutes. To this was added 50 mL of 50% aqueous NaOH solution and the resultant two-phase system was stirred at 80 °C for an additional 90 minutes. After allowing the mixture to cool to room temperature, the reaction was diluted with 500 mL of water and extracted with ethyl ether (3 x 250 mL). The combined organic phases were dried over anhydrous sodium sulfate and concentrated under reduced

pressure. The residue obtained was purified by silica gel chromatography (20% ethyl acetate-hexane) to give 9.7 mCi (16%) of **9** as a white gum. CI-MS ($M+NH_4^+$) 402.

2-[[Hydroxy[[tetrahydro-2-[(octadecyloxy)methyl]-2-furanyl-[2-^{14}C]]methoxy]phosphinyl]oxy]-*N,N,N*-trimethylethaminium hydroxide inner salt, 2

Triethylamine (200 µL, 1.38 mmol) was added in one portion to a stirred solution of **9** (9.7 mCi, 0.942 mmol) and phosphorous oxychloride (97 µL, 1.04 mmol) in 8.0 mL of dichloromethane at 5 °C under a nitrogen atmosphere. The reaction was stirred at 25 °C for 16 hours and then re-cooled to 5 °C. To this was added, in sequence, choline tosylate (540 mg, 1.98 mmol), pyridine (840 µL, 10.4 mmol) and DMAP (25 mg, catalytic). The mixture was stirred at 25 °C for three days. At this point, 12 mL of water was added to the reaction mixture and the solution was heated at reflux for three hours and then concentrated under reduced pressure to a white paste. This was passed through 26 g of Amberlite MB-3 ion exchange resin, eluting with 10% water-THF. The fractions of interest were pooled and concentrated *in vacuo* and the residue was purified by silica gel chromatography (62.5% dichloromethane-31.25% methanol-6.25% water) to give 2.9 mCi (30%) of **2** as a white solid. FAB-MS m/e 550; ^{1}H-NMR (300 MHz, CH_3OD) δ 0.95 (m, 3H), 1.31 (brs, 30H), 1.57 (m, 2H), 1.91 (m, 3H), 3.21 (s, 9H), 3.41 (m, 4H), 3.58 (m, 2H), 3.82 (m, 4H), 4.82 (m, 2H).

2,2,3,3,9,9,10,10-Octamethyl-4,8-dioxa-3,9-disilaundecan-6-one, 11

To a stirred solution of 1,3-dihydroxyacetone dimer, **10**, (15g, 83.3 mmol) and *tert*-butyldimethylsilyl chloride (6.5 g, 375 mmol) in 150 mL of DMF was added imidazole (26 g, 375 mmol) at 25 °C in one portion. The reaction mixture was allowed to stir at room temperature for 18 hours and was then diluted with 500 mL of water. The aqueous suspension was extracted with hexane (3 x 500 mL) and the organic phase was dried over anhydrous sodium sulfate and concentrated under diminished pressure to give 51.3 g (97%) of **11** as a clear oil. CI-MS ($M+NH_4^+$) 336; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.07 (s, 12H), 0.91 (s, 18H), 4.41 (s, 4H).

2,2,3,3,9,9,10,10-Octamethyl-6-[3-(tetrahydro-2*H*-pyran-2-yl)oxypropynyl]-4,8-dioxo-3,9-disilaundecan-6-ol, 12

To a stirred solution of tetrahydro-2-(2-propynyloxy)-2*H*-pyran (20 mL, 145 mmol) in 300 mL of tetrahydrofuran (THF) at -78 °C was added *via* cannula n-butyllithium (1.6 M in hexane, 100 mL, 160 mmol) under a nitrogen atmosphere. The solution was allowed to warm to room temperature and was stirred for one hour, then it was re-cooled to -78 °C. To this was added dropwise a solution of **11** (25 g, 80 mmol) in 50 mL of THF. Following addition, the reaction mixture was allowed to warm to room temperature and was stirred for 18 hours. The reaction was quenched by the addition of 100 mL of saturated ammonium chloride solution and the reaction contents were concentrated under reduced pressure. The residue was partitioned with ethyl ether (2 x 300 mL) and the combined organic phases were concentrated *in vacuo*. The oily residue was purified by silica gel chromatography (15% ethyl acetate-hexane) to give 30.3 g (83%) of **12** as a clear oil. CI-MS ($M+NH_4^+$) 476.

2,2,3,3,9,9,10,10-Octamethyl-6-[3-(tetrahydro-2*H*-pyran-2-yl)oxy-2-propenyl]-(Z)-4,8-dioxa-3,9-disilaundecan-6-ol, 13

Hydrogen gas was passed over a stirred suspension of **12** (10 g, 21.8 mmol) and Lindlar catalyst (50 mg) in 100 mL of hexane for two hours at room temperature. The reaction mixture was suction-filtered through a pad of silica gel and the pad was then washed with 600 mL of dichloromethane. The combined filtrates were concentrated under reduced pressure to give 8.4 g (83%) of **13** as a clear oil. CI-MS ($M+NH_4^+$) 478.

1,1-Bis[(1,1-dimethylethyl)dimethylsilyloxy]-(Z)-2-butenol, 14

To a stirred solution of **13** (8.2 g, 17.8 mmol) in 100 mL of diethyl ether was added in one portion magnesium bromide etherate complex (14 g, 53.4 mmol) at room temperature. The resultant suspension was stirred at 25 °C for one hour and then concentrated under reduced pressure. The residue was purified by silica gel chromatography (30% ethyl acetate-hexane) to give 4.6 g (69%) of the title compound **14** as a clear oil. CI-MS (MH^+) 377.

2,2-Bis[(1,1-dimethylethyl)dimethylsilyloxy]-2,5-dihydrofuran, 15

To a stirred solution of **14** (4.5 g, 11.7 mmol) and methanesulfonylchloride (1.1 mL, 14.0 mmol) in 50 mL of dichloromethane at 5 °C was added dropwise triethylamine (2.5 mL, 17.6 mmol). The mixture was allowed to warm to room temperature and was stirred for 18 hours. At this point, the contents of the reaction vessel were suction-filtered through a pad of silica gel and the pad was washed with an additional 1 L of hexane. The combined filtrates were concentrated *in vacuo* to give 2.2 g (53%) of **15** as a clear oil. CI-MS (MH^+) 359; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.00 (s, 12H), 0.89 (s, 18H), 3.61 (m, 4H), 4.63 (m, 2H), 5.70 (m, 1H), 5.95 (m, 1H).

2,5-Dihydro-2,2-furandimethanol, 16

To a stirred solution of **15** (2.2 g, 6.15 mmol) in 20 mL of THF was added dropwise tetrabutylammonium fluoride (1.0 M solution in THF, 12.3 mL, 12.3 mmol) at room temperature. The reaction mixture was stirred for 30 minutes and then concentrated under reduced pressure. The residue was purified by silica gel chromatography (20% methanol-ethyl acetate) to give 0.25 g (31%) of the title material **16** as a clear gum. CI-MS ($M+NH_4^+$) 148; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.11 (brs, 2H), 3.72 (brs, 4H), 4.75 (m, 2H), 5.78 (dt, 1H, J=9 Hz and 3.5 Hz), 6.12 (dt, 1H, J=9 Hz and 3.5 Hz).

Tetrahydro-2,2-furandimethanol-[*3,4*-^{3}*H*], 17

Tritium gas (2 Ci) was introduced *via* tritium manifold into a reaction vessel containing **16** (50 mg, 0.385 mmol) and 5% palladium on barium sulfate (100 mg) in 500 µL of dichloromethane at 25 °C. The reaction was stirred for 3 hours and then hydrogen gas was bled into the reaction vessel and stirring was continued for an additional 18 hours at room temperature. The mixture was suction-filtered

through a bed of Celite® and the pad was washed with an additional 110 mL of ethanol. To the filtrate was added tetrahydro-2,2-furandimethanol[1] (1.0 g, 7.58 mmol). The resultant solution was determined to contain 470 mCi of **17** upon assay.

Tetrahydro-2-[(octadecyloxy)methyl]-2-furanmethanol-[*3,4-^3H*], 18

A solution consisting of **17** (470 mCi), octadecyl bromide (3.2 g, 9.60 mmol) and tetrabutylammonium hydrogensulfate (3.2 g, 9.42 mmol) in 30 mL of toluene was stirred at 60 °C for 20 minutes. To this was then added 30 mL of 50% aqueous NaOH solution and the resultant two-phase system was stirred at 80-90 °C for an additional two hours. After allowing the mixture to cool to room temperature, the reaction was diluted with 250 mL of water and extracted with ethyl ether (3 x 100 mL). The combined organic phases were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue obtained was purified by silica gel chromatography (20% ethyl acetate-hexane) to give 217 mCi (46% radiochemical yield) of **18** as a clear oil.

2-[[Hydroxy[[tetrahydro-2-[(octadecyloxy)methyl]-2-furanyl-[*3,4-^3H*]]methoxyl]phosphinyl]-oxy]-*N,N,N*-trimethylethaminium hydroxide inner salt, 3

Triethylamine (1.1 mL, 7.80 mmol) was added in one portion to a stirred solution of **18** (217 mCi) and phosphorous oxychloride (0.62 mL, 6.60 mmol) in 35 mL of dichloromethane at 5 °C under a nitrogen atmosphere. The reaction was stirred at 25 °C for 16 hours and then re-cooled to 5 °C. To this was added, in sequence, choline tosylate (3.3 g, 12.0 mmol), pyridine (11 mL, 132 mmol) and DMAP (100 mg, catalytic). The mixture was stirred at 25 °C for 4 days. At this point, 50 mL of water, 8 mL of pyridine and 25 mL of THF was added to the reaction mixture and the solution was heated at reflux for five hours and then concentrated under reduced pressure to a white paste. This was passed through 60 g of Amberlite MB-3 ion exchange resin, eluting with 10% water-THF. The fractions of interest were pooled and concentrated *in vacuo* and the residue was purified by silica gel chromatography (62.5% dichloromethane-31.25% methanol-6.25% water) to give a white gum that was crystallized from dichloromethane-acetone to yield 100.7 mCi (46%) of **3** as a white, granular solid. An additional 53.3 mCi (25%) of **3** was recovered from the mother liquor. FAB-MS m/e 550, ^{1}H-NMR (300 MHz, CD_3OD) δ 0.95 (m, 3H), 1.31 (brs, 30H), 1.57 (m, 2H), 1.91 (m, 3H), 3.21 (s, 9H), 3.41 (m, 4H), 3.58 (m, 2H), 3.82 (m, 4H), 4.28 (m, 2H).

References

1. Houlihan, W.J.; Lee, M.L.; Munder, P.G.; Nemecek, G.M.; Handley, D.A.; Winslow, C.M.; Happy, J.; and Jaeggi, C.- *LIPIDS* **22** : 884 (1987).

2. Tse, F.; and Nickerson, D.- Sandoz Research Institute, unpuplished results.

3. Danheiser, R.L.; Miller, R.F.; Brisbois, R.G.; and Park, S.Z.- *J. Org. Chem.* **55** : 1959 (1990).

4. Adams, J.; and Spero, D.M.- *Tetrahedron* **47** : 1765 (1991).

5. Still, W.C.; Kahn, M.; and Mitra, A.- *J. Org. Chem.* **43** : 2923 (1978).

6. Kim, S.; and Park, J.M.- *Tetrahedron Lett.* **28** : 439 (1987).