JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 353–363.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.676

Research Article

Preparation of labeled bis-amino acid Hydroxyethylamino sulfonamide HIV protease inhibitors

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Summary

Preparation of radiolabeled sulfonamide HIV protease inhibitors, DMP852 and DMP853 is described. The C-14 labeling of DMP852 was accomplished by acylation of the intermediate amine with [1-^{14}C]-chloroacetic anhydride followed by the treatment of the chloroacetyl derivative with methylamine. Due to the rapid loss of the methylglycine moiety during metabolism studies, a route that involved an oxidation-reduction sequence was employed to prepare tritium labeled DMP852. Similar methodology was utilized to prepare tritium labeled DMP853. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: [1-^{14}c]-chloroacetic anhydride; Sodium borotritide; Retroviral protease inhibitors

† This work was completed at Stine-Haskell Research Center, Drug Metabolism and Pharmacokinetics Department, prior to acquisition of DuPont Pharmaceuticals Company by Bristol-Myers Squibb Company.

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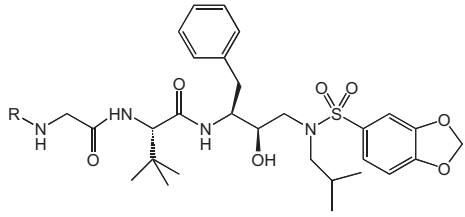
Received 8 May 2002
Revised 25 September 2002
Accepted 17 October 2002

Introduction



R = methyl, DMP852 **1**

R = cyclopropyl, DMP853 **2**

DMP852 **1** and DMP853 **2** are two potent HIV protease inhibitors discovered by Searle scientists.[1] These compounds belong to a class of bis-amino acid hydroxyethylamino sulfonamide derivatives, and were in-licensed from Searle. In order to study their metabolism and pharmacokinetic behavior, radiolabeled versions of the two compounds were required. Selection of a labeling site with peptides like DMP852 and DMP853 presents challenges as one has to consider several factors or may even have to attempt several versions before settling on one that is most appropriate. Preliminary metabolism investigations revealed that incorporation of the label at the terminal amide carbon of DMP852 would be adequate for the purpose.

The C-14 labeled version of DMP852 was prepared by a two-step sequence utilizing [1-^{14}C]-chloroacetic anhydride as the precursor. Detailed metabolism investigations precluded the use of this labeled version due to the loss of the methylglycine portion. An attractive alternative to C-14 labeling appeared to be utilization of an oxidation–reduction sequence to yield tritium labeled DMP852. It was also anticipated that reduction of the ketone obtained by oxidation of DMP852 with sodium borotritide would yield a mixture of diastereomers containing the desired product. This strategy was successfully employed to prepare tritium labeled DMP852 and was later extended to preparation of labeled DMP853.

Results and discussion

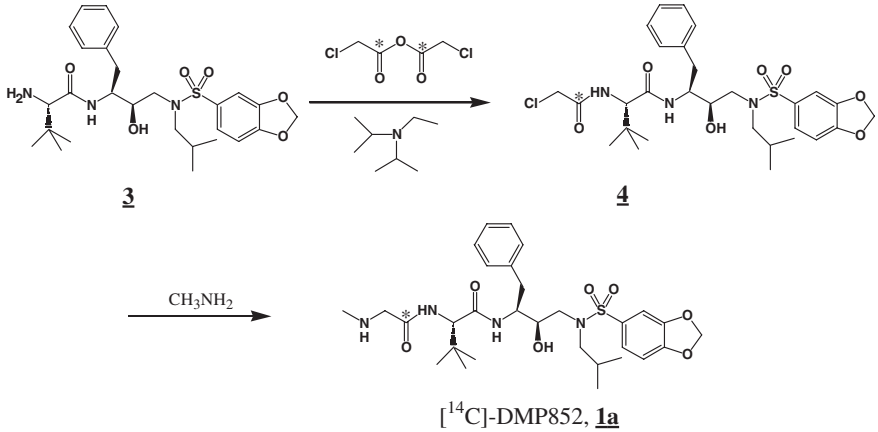
[^{14}C]-DMP852 1a

To generate the labeled compound in a timely manner, we resorted to procedures and conditions that were used to prepare the unlabeled compound (Scheme 1).[1] Chloroacetic anhydride was employed in the original synthesis (Scheme 1), and we used the analogous [1-^{14}C] reagent rather than [1-^{14}C]-chloroacetic chloride because of its lower volatility. Reaction of amine **3** with 1.1 equivalent of labeled chloroacetic anhydride provided the labeled chloroacetyl derivative, which was used in the next step without further purification. Treatment of **4** with excess methylamine followed by crystallization of the hydrochloride salt yielded pure **1a**.

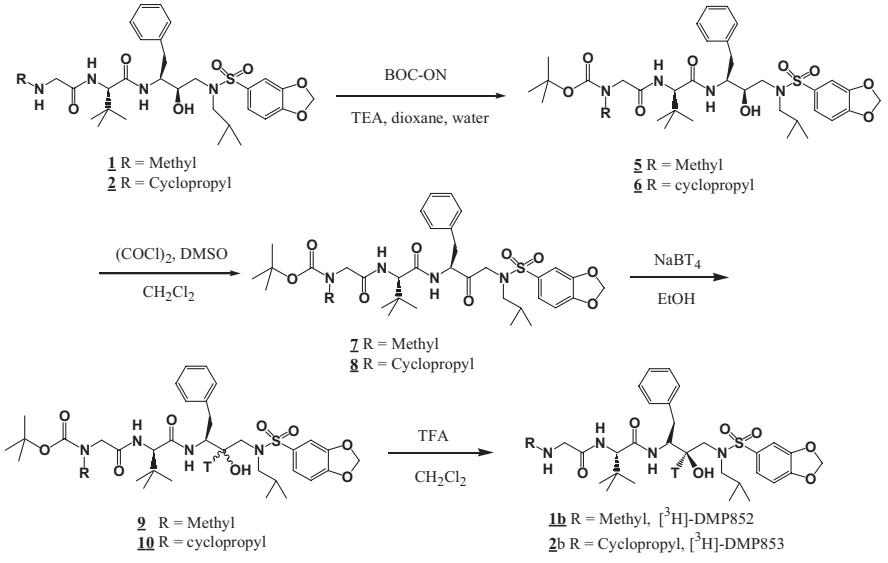
[^{3}H]-DMP852 1b

The oxidation–reduction strategy useful in rapid preparation of tritium labeled compounds containing a secondary alcohol group appeared to be ideally suited to preparation of tritium labeled versions of DMP852 and DMP853 (Scheme 2). Swern oxidation[2,3] of BOC protected DMP852 provided the ketone in excellent yield, and sodium borotritide reduction of the ketone gave a mixture of diastereomeric products, which were separated using HPLC methods.

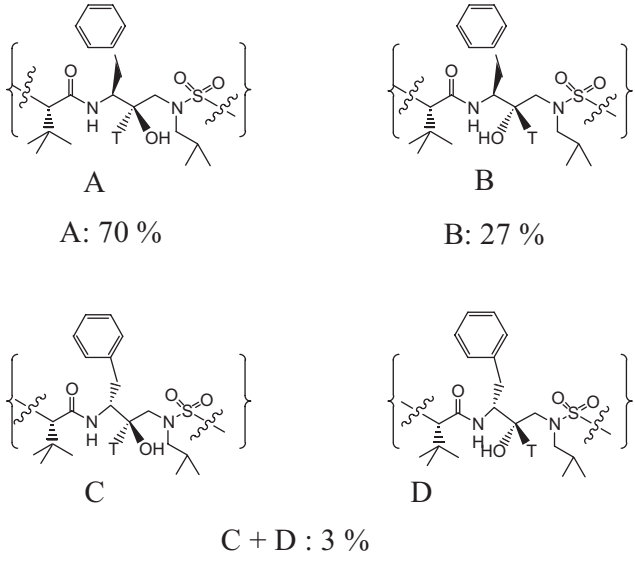
As shown in Scheme 3, in the case of DMP852 the diastereoselectivity during reduction was around 70% in favor of the desired isomer **A**.



Scheme 1.



Scheme 2.



Scheme 3.

Diastereomer **B** was formed to an extent of 27% and the remainder could be attributed to other two diastereomers (**C**, **D**) resulting perhaps from enolization of the ketone before tritiation. Only the desired isomer was separated by HPLC on a reversed-phase column and deprotection

of the isolated compound, followed by further purification, yielded [^{3}H]-DMP852 **1b**, the structure of which was confirmed by ^{1}H-NMR and ^{3}H-NMR analyses, and by mass spectrometry. The specific activity of **1b** was 2.5 Ci/mmol. Similar methodology was used for the synthesis of [^{3}H]-DMP853 **2b**, specific activity 2.8 Ci/mmol.

Two batches of sodium borotritide were used, with specific activity 11.8 Ci/mmol for DMP852 and 14 Ci/mmol for DMP853, respectively. The expected specific activity of the products should have been 2.95 Ci/mmol for DMP852 and 3.5 Ci/mmol for DMP853. Even with an intentional excess of sodium borotritide to mitigate its consumption by moisture, the tritium–proton exchange due to moisture still caused a lowering of specific activity (2.5 Ci/mmol for DMP852 and 2.8 Ci/mmol for DMP853).

In summary, we have described a simple method for the preparation of tritium labeled bis-amino acid hydroxyethylamino sulfonamide HIV protease inhibitors. The majority of highly active compounds in this series have the central unit bearing the hydroxyl group.[1] This labeling methodology is therefore expected to be generally applicable to any other compounds chosen for further progression.

Experimental

^{1}H-NMR and ^{3}H-NMR spectra were obtained from a Bruker 500 spectrometer at 500 and 533 mHz, respectively. Mass spectral analyses were conducted on a Finnigan TSQ-70 or a LCQ spectrometer. Radioactivity determinations were carried out with a Packard liquid scintillation counter, using external standard methods and Ultima Gold as the scintillation fluid. HPLC analyses were performed using a Waters Alliance or HP1100 LC system with a Radiomatic model Flo-One/Beta CR radioactivity detector. The mobile phase consisted of solvent A: 0.05% (v/v) TFA in water, B: 0.05% TFA in acetonitrile. Column: Zorbax RX-C18, 4.6 mm × 25 cm, flow: 1 ml/min, linear gradient elution from 30% B to 90% B in 30 min. TLC was carried out on silica gel 60 F254 plates (Merck). [1–^{14}C]-Chloroacetic anhydride was obtained from ChemSyn Science Laboratories and sodium borotritide was obtained from NEN Life Science Products. Identities of labeled compounds were established by co-chromatography with pure unlabeled compounds.

2 S-N-{(1S,2R)-3-[(1,3-Benzodioxol-5-ylsulfonyl)(isobutyl)amino]-1-benzyl-2-hydroxypropyl}-3,3-dimethyl-2-[(2-chloro-[1-^{14}C]acetyl)amino]butanamide 4

To a stirring solution of **3** (257 mg, 0.45 mmol), diisopropylethylamine (200 μL) in tetrahydrofuran (THF, 3 mL) at 4°C was added dropwise a solution of [1-^{14}C]-chloroacetic anhydride (60 mCi, 0.53 mmol, radiochemical purity 90%) in THF (1 ml). The temperature of the reaction mixture was maintained at 4 – 5°C with continued stirring for another 3 h. The reaction mixture was diluted with ethyl acetate (EtOAc, 10 ml), washed with water (saturated sodium bicarbonate, 5 ml × 2), 5% citric acid in water (5 ml × 2), and brine (5 mL × 2) consecutively. HPLC and TLC (50% EtOAc in heptane v/v) analysis of the EtOAc extract indicated the presence of 75% of the chloroacetyl product. This solution was evaporated to dryness and the residue was used directly in the next step without further purification.

2 S-N-{(1S,2R)-3-[(1,3-Benzodioxol-5-ylsulfonyl)(isobutyl)amino]-1-benzyl-2-hydroxypropyl}-3,3-dimethyl-2-{[2-(methylamino)-[1-^{14}C]acetyl]amino}butanamide 1a

To the residue **4** in THF (5 ml) at 5°C was added methylamine (40% in water, 1 ml) and the mixture was stirred for 6 h. The reaction mixture was diluted with EtOAc (10 ml), washed with saturated sodium bicarbonate in water (5 ml × 2) and brine (5 ml × 2). The EtOAc extract was acidified with concentrated HCl (37%, 0.2 ml) and evaporated to dryness. The residue was crystallized from ethanol (1 ml) and ethyl ether (1 ml) to yield the title product (14.6 mCi, yield 49%). Radiochemical purity, > 99.5% by HPLC.

tert-Butyl 2-{(1S)-1-[({(1S,2R)-3-[(1, 3-benzodioxol-5-ylsulfonyl)(isobutyl)amino]-1-benzyl-2-hydroxypropyl}amino)carbonyl]-2,2-dimethylpropyl}amino-2-oxoethyl(methyl) carbamate 5

To a stirred mixture of **1** (320 mg, 0.50 mmol) in water (1 ml) and 1, 4-dioxane (1 ml) was added triethylamine (TEA, 105 μl) and 2-(*t*-butoxycarbonyloxyimino)-2-phenylacetonitrile (BOC-ON, 136 mg, 0.55 mmol). After stirring at room temperature for 3 h, water (2 ml) was added, the mixture was extracted with ether (3 × 5 ml). The ethereal extract was dried over $MgSO_4$ and filtered. The residue obtained on evaporation was purified by preparative TLC (5:4 v/v hexane:EtOAc) to

give **5** (339 mg, 96%), which was shown by HPLC to be > 99% pure. Mass spectrum (ES+): 704.9 $[M+H]^+$, 648.9 $[M+H—t\text{-Bu}]^+$, 605.2 $[M+H—t\text{-BuOCO}]^+$, and 421.0 (fragment containing the hydroxyl group from cleavage of the center amide bond).

tert-Butyl 2-{(1S)-1-[({(1S,2R)-3-[(1,3-benzodioxol-5-ylsulfonyl)(isobutyl)amino]-1-benzyl-2-oxopropyl}amino)carbonyl]-2,2-dimethylpropyl}amino-2-oxoethyl(methyl) carbamate 7

Oxalyl chloride (43 µl, 0.49 mmol) in CH_2Cl_2 (1 ml) was placed in a 5 ml flask at –78°C, DMSO (70 µl, 0.98 mmol) in CH_2Cl_2 (0.5 mL) was added dropwise, and stirring was continued for 2 more minutes. Upon addition of **5** (289 mg, 0.28 mmol) in CH_2Cl_2 (1 mL) over 5 minutes, a slurry was formed. The reaction mixture was stirred at −78°C for 15 more minutes, triethylamine (279 µL, 2 mmol) was added and stirring was continued at −78°C for a further 5 min. The reaction mixture was allowed to warm to room temperature, water (2 ml) was added, the CH_2Cl_2 layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 2 ml). The CH_2Cl_2 extracts were combined and washed with 0.2% HCl (2 ml), 5% $NaHCO_3$ (2 ml), water (2 ml), dried over $MgSO_4$, filtered and evaporated to give a solid **7** (260 mg, 90%, >99% pure by HPLC). Mass spectrum (ES+): 702.8 $[M+H]^+$, 685.1 $[M+H—H_2O]^+$, 646.9 $[M+H—t\text{-Bu}]^+$, 603.2 $[M+H—t\text{-BuOCO}]^+$, and 419.1(fragment containing the keto group from cleavage of the center amide bond). ^{1}H-NMR (DMSO-d_6): δ (ppm) 7.20 (m, 7 H), 7.02 (d, 1 H), 6.15 (s, 2 H), 4.55 (m, 1 H), 4.28 (m, 2 H), 3.60-4.00 (m, 3 H), 3.05 (m, 2 H), 2.89 (m, 1 H), 2.73 (m, 4 H), 1.60 (m, 1 H), 1.31 (s, 9 H), 0.85 (s, 9 H), 0.71 (m, 6 H).

Reduction of 7 by $NaBH_4$

To a stirred solution of **7** (112 mg, 0.16 mmol) in ethanol (4 mL), was added dropwise a solution of 0.04 M $NaBH_4$ in ethanol (0.04 mmol, 1 mL). The reaction mixture was stirred at room temperature overnight. HPLC analysis of an aliquot of the reaction mixture showed complete consumption of **7** and formation of BOC-DMP852 and its diastereomers. Ethanol was removed by distillation under vacuum. The residue was redissolved in 50% B, any excess of $NaBH_4$ was quenched. The crude was purified on a Zorbax RX C18 (9.4 mm x 25 cm, flow rate: 4 ml/min, 254 nm, mobile phase: 50% B). Compound **5** eluted at

14.5 min and was baseline separated from other diastereomers. Lyophilization of the pooled fractions containing **5** provided pure compound, 75 mg, yield 66%.

Deprotection of 5

To **5** from the previous reaction (10 mg, 0.014 mmol) was added 30% TFA in CH_2Cl_2 (1 ml), and the solution was stirred at room temperature for 4 hours. HPLC analysis showed completion of the reaction. The solvent was evaporated via vacuum distillation. The residue was purified on a semi-preparative column to give **1**, 5.3 mg, 65%. (Zorbax RX C18, 9.4 mm × 25 cm, flow rate: 4 ml/min, 254 nm, gradient elution in 30 min from 10% B to 90% B, t_R of **1**, 8 min).

tert-Butyl 2-{(1S)-1-[({(1S,2R)-3-[(1,3-benzodioxol-5-ylsulfonyl)(isobutyl)amino]-1-benzyl-2-hydroxy-2-[^{3}H]-propyl}amino)carbonyl]-2,2-dimethylpropyl}amino-2-oxoethyl (methyl) carbamate 9 (Reduction of 7 by NaB[^{3}H]$_4$)

To **7** (10 mg) in a reaction vial was added NaB[^{3}H]$_4$ (100 mCi) in absolute ethanol (2 ml), the vial was then sealed and stirred at room temperature for 4 h. HPLC showed formation of BOC protected [^{3}H]-DMP852 and three other diastereomers (see Results and Discussion). Ethanol was removed via vacuum distillation. The residue was dissolved in 50% B (6 ml), and purified on a Zorbax RX C18 column (9.4 mm × 25 cm, flow rate: 4 ml/min, 254 nm, mobile phase: 50% B). Fractions containing pure BOC protected [^{3}H]-DMP852 (t_R 14.5 min) were combined and utilized in the next step without further processing. Total activity was 16.4 mCi.

(2S)-N-{(1S,2R)-3-[(1,3-Benzodioxol-5-ylsulfonyl)(isobutyl)amino]-1-benzyl-2-hydroxy-2-[^{3}H]-propyl}-3,3-dimethyl-2-{[2-(methylamino)acetyl]amino}butanamide 1b

The pooled fractions containing **9** were concentrated by vacuum distillation, followed by addition of 30% TFA in CH_2Cl_2 (1 mL). After 2 hours, the solvent was removed by evaporation with a gentle stream of N_2. The residue obtained was redissolved in 30% acetonitrile (2 mL), and chromatographed on a Zorbax RX C18 column (9.4 mm × 25 cm, flow rate: 4 ml/min, 254 nm, linear gradient elution from 10% B to 90% B in 30 min). Radiochemical purity of the combined fractions was 96%.

The combined fractions were then lyophilized and rechromatographed on the same semi-preparative column (flow rate: 4 ml/min, 254 nm, gradient elution from 10% B, to 70% B in 30 min, t_R 11.4 min). Fractions collected were lyophilized again to give **1a** (5 mCi, radiochemical purity, > 99.5%), which was stored in absolute ethanol (5 ml). Radiochemical yield 5% (from $NaB[^3H]_4$, reflected higher losses during purification). Specific activity as determined by HPLC/UV assay was 2.5 Ci/mmol. ^{3}H-NMR (proton decoupled, DMSO-d_6): singlet at 4.0 ppm.

tert-Butyl 2-{(1S)-1-[({(1S,2R)-3-[(1,3-benzodioxol-5-ylsulfonyl)(isobutyl)amino]-1-benzyl-2-hydroxypropyl}amino)carbonyl]-2,2-dimethylpropyl}amino-2-oxoethyl (cyclopropyl) carbamate 6

To the free base generated from **2** (HCl salt, 513 mg, 0.77 mmol) was added water (2 ml), dioxane (2.5 mL), triethylamine (162 µl), followed by the addition of 2-(*t*-butoxycarbonyloxyimino)-2-phenylacetonitrile (BOC-ON, 208 mg, 0.85 mmol). After stirring at room temperature for 4 h, water (5 ml) was added, the aqueous solution was extracted with EtOAc and the EtOAc extract was dried over $MgSO_4$. The crude product obtained by evaporation of EtOAc was purified by preparative TLC (1:2 v/v EtOAc:hexane), to afford > 98% pure **6** (477 mg, 85%). ^{1}H-NMR (DMSO-d_6): δ 7.32 (d, 1 H), 7.27 (s, 1 H), 7.15 (m, 5 H), 7.05 (d, 1 H), 6.15 (s, 2 H), 4.95 (d, 1 H), 4.17 (d, 1 H), 4.00 (m, 1 H), 3.75 (bs, 1 H), 3.58 (m, 1 H), 3.25 (m, 1 H), 2.95 (m, 3 H), 2.80 (m, 1 H), 2.53 (m, 1 H), 1.92 (m, 1 H), 1.30 (s, 9 H), 0.8 (m, 15 H), 0.55 (m, 4 H). Mass spectrum (ES+), *m/z*: 753.3 $[M + Na]^+$, 697.1 $[M + Na - t\text{-Bu}]^+$, 653.4 $[M + Na - t\text{-BuOCO}]^+$.

tert-Butyl 2-({(1S)-1-[({(1S,2R)-3-[(1,3-benzodioxol-5-ylsulfonyl)(isobutyl)amino]-1-benzyl-2-oxopropyl}amino)carbonyl]-2,2-dimethylpropyl}amino-2-oxoethyl (cyclopropyl) carbamate 8

Following the procedure described for the preparation of **7**, **8** was synthesized in 90% yield. Mass spectrum (ES+), *m/z*: 751.3 $[M + Na]^+$, 695.1 $[M + H—t\text{-Bu}]^+$, 651.3 $[M + H—t\text{-BuOCO}]^+$.

Reduction of 8 by $NaBH_4$

By analogy with sodium borohydride reduction of **7**, **8** yielded BOC protected DMP853 (69%), another major diastereomer (28%) and a

pair of other diastereomers (4%). Deprotection of the mixture with TFA in CH_2Cl_2 followed by preparative HPLC purification yielded **2** (Vydac protein peptide C18, 9.4 mm × 25 cm, flow rate: 4 ml/min, 254 nm, mobile phase: 40% acetonitrile in 0.2% aqueous TFA).

tert-Butyl 2-{(1S)-1-[({(1S,2R)-3-[(1,3-benzodioxol-5-ylsulfonyl)(isobutyl)amino]-1-benzyl-2-hydroxy-2-[^{3}H]-propyl}amino)carbonyl]-2,2-dimethylpropyl}amino-2-oxoethyl (cyclopropyl) carbamate 10 (Reduction of 8 by NaB[^{3}H]$_4$)

Following the same reaction conditions for reduction of **7** by NaB[^{3}H]$_4$ (100 mCi), HPLC assay showed formation of BOC protected [^{3}H]-DMP853 (68%), another major diastereomer (28%) and a pair of other diastereomers (4%). Ethanol was removed via vacuum distillation to give **10** (28.2 mCi). Compound **10** was used directly in the next step without further purification.

(2S)-N-{(1S,2R)-3-[(1,3-Benzodioxol-5-ylsulfonyl)(isobutyl)amino]-1-benzyl-2-hydroxy-2-[^{3}H]-propyl}-3,3-dimethyl-2-{[2-(cyclopropylamino)acetyl]amino} butanamide 2b

A solution of 30% TFA in CH_2Cl_2 (1 ml) was added to **10** (28.2 mCi) and stirred at room temperature for 2 h. HPLC assay showed completion of the reaction. Dichloromethane was removed via vacuum distillation, the residue was redissolved in 40% acetonitrile in 0.2% aqueous TFA (5 ml), chromatographed on a Vydac protein peptide C18 column (9.4 mm × 25 cm, flow rate: 4 ml/min, 254 nm, mobile phase: 40% acetonitrile in 0.2% aqueous TFA, t_R, 5 min). Combined fractions were lyophilized to give **2b** (10 mCi, 10% from NaB[^{3}H]$_4$), which was stored in absolute ethanol (10 mL). Specific activity was determined by HPLC/UV assay to be 2.8 Ci/mmol. ^{3}H-NMR (proton decoupled, DMSO-d_6): singlet at 4.0 ppm.

Acknowledgements

The authors thank Gregory Harris for providing DMP852 and DMP853 samples, Abdul Mutlib, John Shockcor and Hao Chen for mass spectral data and NMR spectral data.

References

1. Getman DP, Descrescenzo GA, Freskos JN, *et al.* (GDS Searle & Co., USA). PCT Int. Appl., 255 pp. CODEN: PIXXD2. WO 9628464 A1 960919. Application: WO 96-US2685 960307. Priority: US 95-402450 950310; US95-479071 950607 CAN 126: 8710.
2. Mancuso AJ, Brownfain DS, Swern D. *J Org Chem* 1979; **44:** 4148-4150.
3. Swern oxidation of DMP852 without protection of the amino group resulted in a mixture of *N*-dimethyl sulfonium salts.

