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An Efficient Synthesis of *N*-Hydroxy-*N'*-[4-^{3}H]phenyloctanediamide ([4-^{3}H]SAHA), a Potent Cytodifferentiating Agent

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SUMMARY

We have developed an efficient synthesis of *N*-hydroxy-*N'*-[4-^{3}H]phenyloctanediamide ([4-^{3}H]SAHA) from the monoester of suberanilic acid. The starting material, a monoester of suberoyl chloride, was condensed with 4-bromoaniline in the presence of a base to give the 4-bromoanilide of monoethyl suberate in 92% yield. On further treatment with methanolic hydroxylamine hydrochloride and sodium methoxide, this compound gave 4-bromosuberanilohydroxamic acid in 94% yield. Catalytic tritium exchange of bromine using Pd/C in a tritium gas atmosphere resulted in [4-^{3}H]SAHA with a specific activity of 2.49 Ci/mmol. From the key intermediate, 4-bromoanilide of monoethyl suberate by a non-catalytic tritium exchange reaction, we have also prepared [4-^{3}H]SAHA with high specific activity of 27.5 Ci/mmol.

Key words: Synthesis, *N*-hydroxy-*N'*-[4-^{3}H]phenyloctanediamide ([4-^{3}H]SAHA), Cytodifferentiating agent

Introduction

The use of cytodifferentiating agents has opened new avenues for preventing the progression of cancer. A prototype of such agents is the hybrid-polar compound hexamethylene bisacetamide (HMBA) which induces terminal differentiation and apoptosis in HL-60 cells (1-3). Compounds structurally related to, but more potent than HMBA, have been described in the literature. Breslow *et al.* (4) who have identified a series of subaric acid derivatives that are 3 orders of magnitude more potent in differentiating cultured leukemia cells than HMBA. Two functional groups in subaric acid derivatives are

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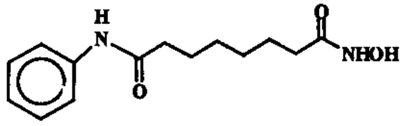
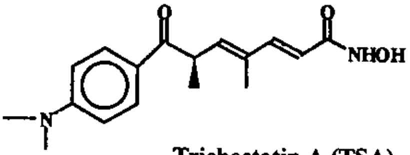
separated by a six-member methylene bridge (4). One such compound, *N*-hydroxy-*N'*-phenyloctanediamide (suberanilohydroxamic acid [SAHA]), is 2000 times more potent than HMBA (5). SAHA is structurally related to trichostatin A (TSA), a natural product isolated from *Streptomyces hygroscopicus* that was initially used as an antifungal antibiotic (6).



N-Hydroxy-N'-phenyloctandiamide (SAHA)



Trichostatin A (TSA)

Like TSA, SAHA is also an *in vitro* inhibitor of histone deacetylase activity (5). At 10^{-4} M concentration SAHA completely inhibited cell proliferation in rat prostate cells *in vitro* (7). At 10^{-6} M concentration of SAHA, cells resumed their usual morphology but proliferated at a slower rate than the control cells. These observations suggest that SAHA holds promise as a potential cancer therapeutic agent.

In addition to the *in vitro* studies described above, Cohen *et al.* (8) have recently provided evidence that SAHA as a dietary supplement inhibits *N*-methylnitrosourea-induced mammary tumors in rats. Thus, SAHA can also inhibit the growth of solid tumors *in vivo* (8). The tissue specificity, metabolic pathways, and the specific metabolites of SAHA which may or may not exert the biological effect need to be explored. Therefore it was the purpose of this study to synthesize radiolabeled SAHA with high specific activity for determining the tissue distribution and metabolism of SAHA in intact animals. This communication describes an efficient synthesis of *N*-hydroxy-N'-[4-^{3}H]phenyloctanediamide ([4-^{3}H]SAHA) developed by us with catalytic as well as non-catalytic tritiation procedures.

Materials and Methods

General:

NMR spectra were determined in $CDCl_3$ on a Bruker AM 360 WB spectrometer. Chemical shifts are expressed in ppm downfield from tetramethylsilane. MS were run on a

Hewlett-Packard Model 5988A instrument and high resolution MS on a Finnigan Mat 95 instrument at the Mass Spectrometry Service, University of Minnesota, MN. Thin-layer chromatographic separations were done on aluminum-supported pre-coated silica gel plates from EM Industries (Gibbstown, NJ). Ethylhydrogen suberate was purchased from Lancaster Synthesis Inc. (Windham, NH). Monoethyl ester of subaryl chloride (2) was prepared in quantitative yield by reacting ethylhydrogen suberate with oxalyl chloride in methylene chloride. Most starting materials were obtained from Aldrich Chemical Co. (Milwaukee, WI).

Methods:

4-Bromoanilide of monoethyl suberate (3): To a cold stirring mixture of 4-bromoaniline (1) (7.38 g, 43 mmol) with triethylamine (10 g, 99 mmol) in dry methylene chloride (100 ml), a solution of monoethyl ester of subaryl chloride (2) (7.32 g, 33 mmol) in methylene chloride (50 mL) was added dropwise in the course of 1 h. The triethylamine hydrochloride salt precipitated, and stirring was continued for 16 h at room temperature. The reaction mixture was poured into water and extracted with EtOAc (3 x 50 mL). The combined organic fractions were dried ($MgSO_4$), filtered, and evaporated, and the residue was purified by chromatography on a silica gel column with elution by EtOAC : hexane (1:1) to yield compound 3 (13.58 g, 92%) as a white solid: mp 104-105°C; ^{1}H NMR δ 7.20-7.45 (m, 5H, aromatic and NH), 4.12 (dd, 2H, OCH_2, J = 7.16 Hz, J = 7.15 Hz), 2.34 (t, 2H, CO-CH_2, J = 7.39 Hz), 2.30 (t, 2H, NH-CO-CH_2, J = 7.31 Hz), 1.77-1.59 (m, 4H, CH_2-CH_2), 1.4-1.32 (m, 4H, CH_2-CH_2), 1.25 (t, 3H, CH_3, J = 7.16 Hz), CI-MS m/e (relative intensity) 355 and 357 (M^+, 40), 310 and 312 (M^+-OC_2H_5, 40), 171 and 173 (40), 139 (50), 83 (58); High resolution CI-MS: Calcd. for $C_{16}H_{22}BrNO_3$, 355.0782 and 357.0762; found, 355.0782 and 357.0790.

***N*-Hydroxy-*N'*-[4-Br]phenyloctanediamide** (4): To a solution of hydroxylamine hydrochloride (1.38 g, 20 mmol) in MeOH (25 mL), 1 mg of phenolphthalein and then

$NaOCH_3$ (1.62 g, 30 mmol) was added. This mixture was stirred for 30 min at room temperature. When sodium chloride precipitated, compound **3** (3.56 g, 10 mmol) was added. The reaction mixture was stirred for an additional 16 h at room temperature and then quenched with 50 mL H_2O and glacial acetic acid (4 mL). Stirring was continued for 1 h and the resulting precipitate was filtered, and rinsed with water. The solid was dried at room temperature to yield **4** (3.22 g, 94%) as a white solid: mp, 182-184 °C; ^1H NMR (DMSO) δ 10.33 (s, 1H, NH), 9.99 (s, 1H, NH), 8.66 (s, 1H, OH), 7.57 (d, 2H, aromatic, J = 8.84 Hz), 7.46 (d, 2H, aromatic, J = 8.83 Hz), 2.29 (t, 2H, CO-CH2, J = 7.42 Hz), 1.93 (t, 2H, NH-CO-CH2, J = 7.33 Hz), 1.56 (t, 2H, C-CH2-C, J = 6.49 Hz, J = 7.13 Hz), 1.48 (t, 2H, C-CH2-C, J = 7.01 Hz, J = 6.72 Hz), 1.2-1.3 (m, 4H, CH2-CH2), MS m/e (relative intensity) 342 and 343 (M^+, 60), 310 and 312 (20), 197 and 199 (10), 172 and 174 (50); High resolution CI-MS: Calcd. for $C_{14}H_{19}BrN_2O_3$, 342.0579 and 344.0558; found, 342.0579 and 344.0513.

[4-^{3}H]Anilide of monoethyl suberate (5): Compound **3** was reduced by cataytic tritium exchange reaction using Pd/C in a tritium gas atmosphere at 40 psi. This procedure was performed at Amersham Pharmacia Biotech Inc. (Piscataway, NJ). The specific activity of [4-^{3}H]anilide of monoethyl suberate (**5**) obtained by this procedure was 16.61 Ci/mmol. The total yield of [4-^{3}H]anilide of monoethyl suberate (**5**) was 300 mCi per tritiation cycle.

***N*-Hydroxy-*N'*-[4-^{3}H]phenyloctanediamide (6):** To a solution of hydroxylamine hydrochloride (1 mg, 0.02 mmol) in MeOH (2 mL), 0.1 mg of phenolphthalein and then $NaOCH_3$ (1.62 mg, 0.03 mmol) was added. This mixture was stirred for 30 min at room temperature. Sodium chloride precipitated and compound **5** (2.80 mg, 0.01 mmol, 25 mCi, 2.49 Ci/mmol) was added. The mixture was then stirred for 16 h at room temperature and quenched with H_2O (5 mL) and glacial acetic acid (0.4 mL). Stirring was continued for 1 h and the resulting precipitate was filtered and rinsed with water. Upon drying at room temperature **6** was obtained as a white solid. The specific activity of compound **6** was 2.49 Ci/mmol as determined by its UV extinction coefficient at 238 λ (ε 33200). The total radiochemical yield of the reaction was 20.4 mCi (81.6%).

***N*-Hydroxy-*N'*-[4-^{3}H]phenyloctanediamide ([4-^{3}H]SAHA) (6)**: Alternatively, compound **6** was obtained by exchanging bromine in compound **4** for tritium using a non-catalytic exchange procedure (9, 10). *N*-Hydroxy-*N'*-[4-^{3}H]phenyloctanediamide ([4-^{3}H]SAHA) was separated from unreacted 4-bromoSAHA by High performance liquid chromatography (HPLC, Figure 1) on a C-18 Vydac analytical column in conjunction with a Gilson UV detector (Middleton, WI) at 254 nm and radioactivity was quantitated with a Flow One/β-ram radioactive flow detector (Inus Systems, Inc., Tampa, FL). Following gradient program was used: 100% water (containing 0.1% trifluoroacetic acid) to 100% methanol in 40 minutes at a flow rate of 1mL/min. The retention time of [4-^{3}H]SAHA was 12 min. The

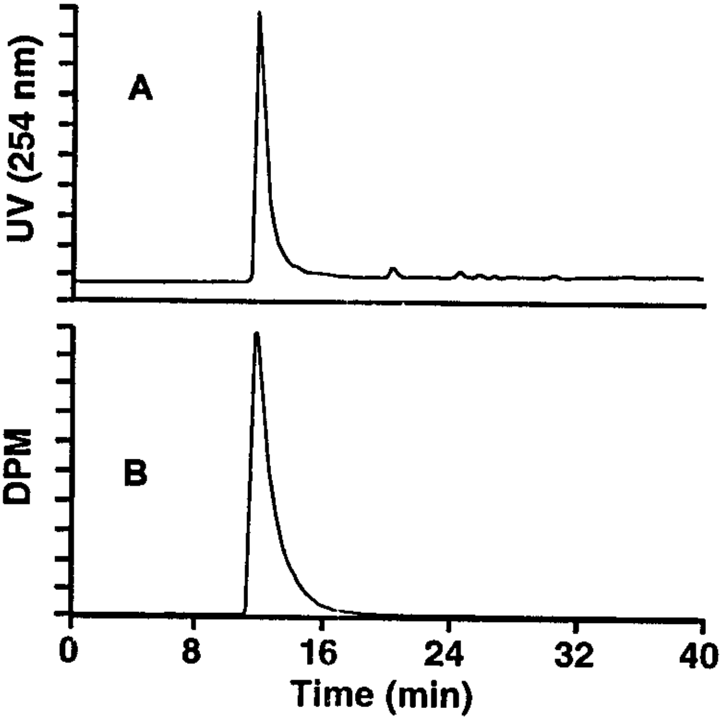
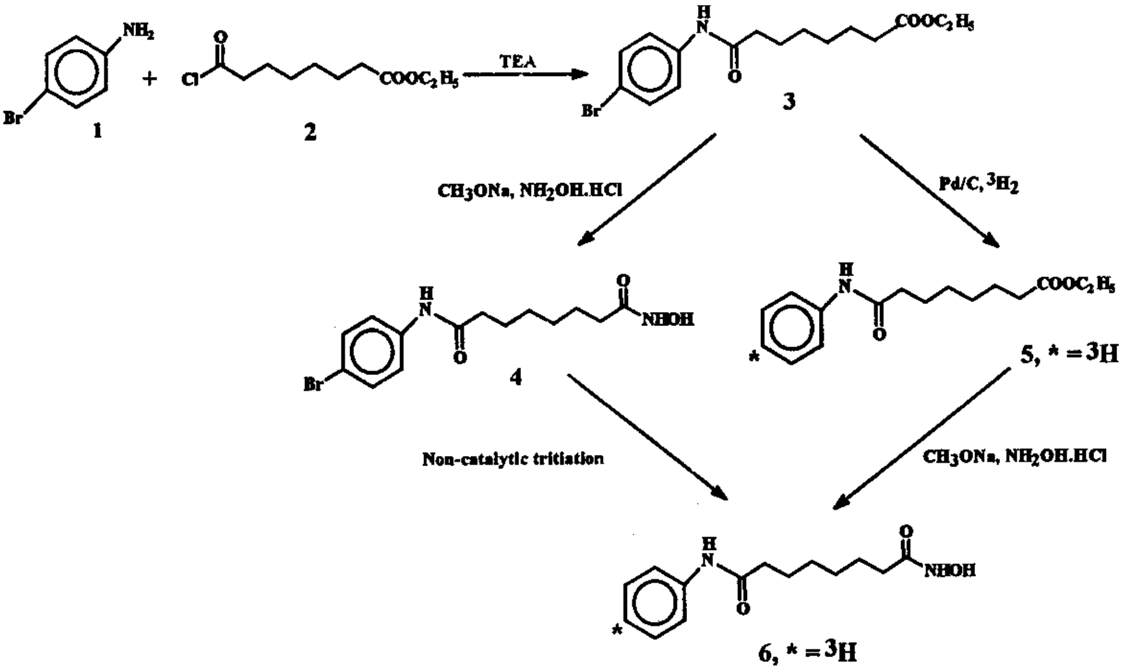


Figure I: HPLC profile of (A) SAHA and (B) [4-^{3}H]SAHA

specific activity of [4-^{3}H]SAHA as determined by its UV extinction coefficient at 238 λ (ε 33200) was 27.5 Ci / mmol and its yield was 0.25 mCi per tritiation cycle.

Discussion

An efficient synthesis of *N*-Hydroxy-*N'*-[4-^{3}H]phenyloctanediamide ([4-^{3}H]SAHA) (6) (scheme I) was developed by modifying a synthetic procedure by Stowell *et al.* (11). In this synthesis the monoester of subaryl chloride served as the starting material; therefore, the formation of dianilide as a side product was eliminated. The monoester of subaryl chloride was condensed with 4-bromoaniline in the presence of triethylamine to give the anilide 3 in 92% yield. Compound 3 when treated with methanolic hydroxylamine hydrochloride and sodium methoxide yielded 4-bromoSAHA (4) in 94% yield. Compound 4 was tritiated by a non-catalytic exchange procedure with tritium gas (9, 10). As a control, cold SAHA was



Scheme I: Synthesis of*N*-hydroxy-*N'*-[4-^{3}H]phenyloctanediamide (6)

tritiated under identical conditions. The [4-^{3}H]SAHA obtained from both bromosubstituted and 'cold' SAHA was purified by reverse phase HPLC on a Vydac analytical column with detection by a Flow One/β-ram radioactive flow (as shown in figure 1). The specific activity of [4-^{3}H]SAHA was determined on the basis of the extinction coefficient of SAHA, at 238 λ (ε =33200). We determined that tritiated SAHA obtained from the bromosubstituted precursor **4** had a specific activity of 27.5 Ci/mmol which is close to the theoretical specific activity of 28.8 Ci/mmol for a compound containing a single tritium atom per molecule. In contrast, tritiated SAHA obtained from 'cold' SAHA had a specific activity of only 0.5-1 Ci/mmol. This suggests that, under our experimental conditions, bromine for tritium exchange in [4-Br]SAHA was significantly more efficient than hydrogen. We therefore conclude, that the tritiated compound obtained from bromosubstituted SAHA is predominantly [4-^{3}H]SAHA (**6**).

In another experiment, the tritium exchange at 40 psi using Pd/C under tritium gas atmosphere with 4-bromoanilide of monoethyl suberate (**3**) was performed (Amersham Pharmacia Biotech Inc., Piscataway, NJ) to give compound **5**. The total radiochemical yield of the anilide **5** was 300 mCi per cycle leading to a specific activity of 16.61 Ci/mmol. Anilide **5** when reacted with hydroxylamine hydrochloride using the method reported by Stowell *et al.* (11) gave [4-^{3}H]SAHA (**6**). Further HPLC purification using C-18 reverse phase Vydac analytical column yielded **6**. The specific activity of compound **6** was found to be 2.49 Ci/mmol on the basis of extinction coefficient for SAHA at 238 λ (ε =33200). The total radiochemical yield of compound **6** was 20.4 mCi (81.6%).

The non-catalytic tritium exchange rection gave [4-^{3}H]SAHA with the desired high specific activity and the tritium moiety was introduced at the last stage. The [4-^{3}H]SAHA with its high specific activity will be highly useful for *in vivo* examination of metabolism, tissue distribution, and tissue specificity in animal models. The catalytic tritiation, a more common procedure leads to [4-^{3}H]SAHA with lower specific activity. This procedure is useful for compounds that are more stable at higher tritium gas pressure.

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