Heterocyclic Letters
Vol. 12| No.1|13-18|November -January|2022
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

PREPARATION OF A RESVERATROL-ASCORBIC ACID MEM CONJUGATE

Rasidul Amin* and David A. Hunt**

* *Symmetry Biosciences, 112 Nova Drive, Suite #1*
Morrisville, NC 27560
***Department of Chemistry, The College of New Jersey, 2000 Pennington Road*
Ewing, NJ 08628
il: hunt@tcnj.edu

Abstract: The preparation of a resveratrol-ascorbic acid conjugate in support of pharmacodynamic and pharmacokinetic studies has been achieved through a seven-step convergent synthesis.

Keywords: Resveratrol, ascorbic acid, conjugation

Introduction

Ascorbic acid is well known as a dietary supplement and biological antioxidant.[1,2] Additionally, other possible applications include treatment for glaucoma, preventing cataracts, preventing gallbladder disease, dental cavities (caries), constipation, Lyme disease, boosting the immune system, heat stroke, hay fever, asthma, bronchitis, cystic fibrosis, infertility, diabetes, chronic fatigue syndrome (CFS), autism, collagen disorders, arthritis and bursitis, back pain and disc swelling, cancer, and osteoporosis. Much of the ascorbic acid in the body is concentrated in the brain[3] and is transported in the oxidized form into the brain through the blood-brain barrier *via* glucose transporters.[4]

Resveratrol is a phytoalexin found in highest concentration in peanuts and red grapes and has been the focus of multiple studies pertaining to possible health benefits including serving as a chemotherapeutic agent for certain cancers.[5,6]

One of the potential uses for both ascorbic acid[7] and resveratrol[8] is for the treatment of cognitive disorders, more specifically Alzheimer's Disease (AD). Alzheimer's Disease is a debilitating disease for which there is no known cure and to date only palliative treatments are available. To date, there are several competing hypotheses for the origination of the disease. One of the issues with treatment of any CNS (central nervous system) disorder is transport of a potential therapeutic agent into the brain. The brain is protected by a membrane known as the blood-brain barrier (BBB). The blood-brain barrier is a separation of circulating blood from the brain extracellular fluid in the CNS. It occurs along all capillaries and consists of tight junctions

around the capillaries that do not exist in normal circulation. Endothelial cells restrict the diffusion of microscopic objects (e.g., bacteria) and large or hydrophilic molecules into the cerebrospinal fluid (CSF) while allowing the diffusion of small hydrophobic molecules (O_2, CO_2, hormones). Cells of the barrier actively transport certain polar metabolic products such as glucose across the barrier with specific proteins. This barrier also includes a thick basement membrane and astrocytic endfeet. Transport of many putative therapeutic agents across the BBB have proven problematic. Therefore, there is a need to develop both compounds and methods for the treatment of certain CNS disorders with compounds that can either move across the blood-brain barrier or shuttle therapeutic molecules across the blood-brain barrier.

During the course of a drug transport study, we required the regioselective preparation of a resveratrol-ascorbic acid conjugate as a potential treatment for Alzheimer's disease, heretofore unknown.

Experimental

General

^{1}H NMR (300 MHz) data were obtained from a Varian Mercury 300MHz nuclear magnetic resonance spectrometer referencing tetramethylsilane. Preparative HPLC was performed on a Teledyne ISCO system using a gold column (125 g cartridge). Analytical HPLC analysis was performed on a Chemstation 1100 LC System using a Phenomenex Luna C18-2 column. All reagents were obtained from commercial sources and were used with further purification.

Synthesis of 3,5-bis((2-methoxyethoxy)methoxy)benzaldehyde (10): A 250-mL, three neck round bottom flask was charged with 3,5-dihydroxybenzaldehyde (**9**) (5 g, 36.2 mmol, 1 equiv), anhydrous dichloromethane (50 mL, 5 vol) and diisopropylethyl amine (DIPEA, 2 equiv). The mixture was cooled to 0-5 ºC and 2-methoxyethoxymethyl chloride (MEMCl, 2 equiv) was added. The reaction mixture was allowed to warm at room temperature stirred for 16 hours under nitrogen. TLC indicated the consumption of the starting material. The mixture was quenched with water (50 mL) and layers separated. The aqueous layer was extracted with DCM (50 mL) and the combined organics were dried over magnesium sulfate, filtered and concentrated. Silica column purification using 20-50% ethyl acetate/hexanes as the eluent afforded product **10** as a light brown oil (11.4 g, >99%). ^{1}H NMR (300 MHz; $CDCl_3$) δ 9.87 (s, 1H, -CHO); 7.20 (t, 2 ArH, J = 2.1 Hz); 6.96 (t, 1 ArH, J = 2.1 Hz); 5.27 (s, 4H, -OCH_2O-); 3.79 (t, 4H, -O**CH$_2$**CH_2O-, J = 4.8 Hz); 3.54 (t, 4H, -OCH_2**CH$_2$**O-, J = 4.8 Hz); 3.35 (s, 6H, -OCH_3).

Preparation of 1,3-bis((2-methoxyethoxy)methoxy)-5-vinylbenzene (11): A 500-mL, three neck round bottom flask was charged with methyltriphenylphosphonium bromide (14.1 g, 39.5 mmol, 1.1 equiv) and anhydrous tetrahydrofuran (200 mL). Sodium tert-butoxide (3.81 g, 39.5 mmol, 1.1 equiv) was added and the mixture refluxed for 1 hour. The mixture was cooled to room temperature and **10** (11.3 g, 35.9 mmol, 1 equiv) added. The mixture was heated to reflux and stirred for 16 hours. TLC analysis indicates complete consumption of compound **10**. The reaction mixture was cooled to room temperature, diluted with water (200 mL) and extracted with ethyl acetate (2 x 100 mL). The combined organics were washed with brine (100 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude was purified by silica column using 10-30% ethyl acetate/hexanes as the eluent to afford product **11** as a light brown oil (10.6 g, 95%). ^{1}H NMR (300 MHz; $CDCl_3$) δ 6.79-6.58 (m, 4H, ArH, vinylic CH); 5.76 (d, 1 vinylic H, J = 18 Hz); 5.26 (s with imbedded multiplet, 5H, -OCH_2O-;

vinylic CH); 3.83 (t, 4H, -O**CH₂**CH_2O-, *J* = 4.8 Hz); 3.57 (t, 4H, -OCH_2**CH_2**O-, *J* = 4.8 Hz); 3.38 (s, 6H, -OCH_3).

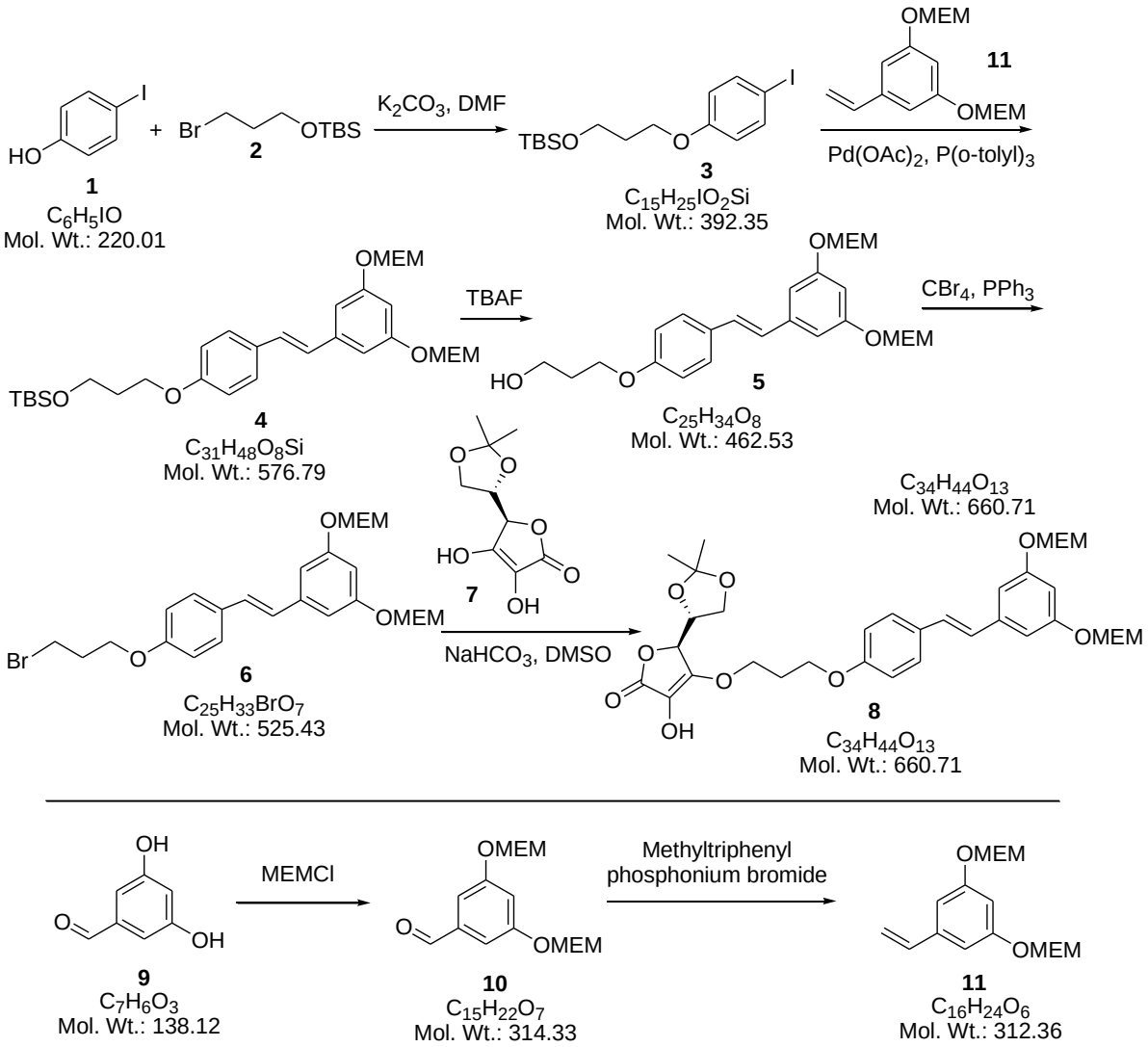
Synthesis of *tert*-butyl(3-(4-iodophenoxy)propoxy)dimethylsilane (3): A 500-mL, three neck round bottom flask was charged with *p*-iodophenol **(1)** (10 g, 45.4 mmol, 1 equiv), potassium carbonate (13.8 g, 99.9 mmol, 2.2 equiv) and anhydrous N,N-dimethylformamide (100 mL, 10 vol) then heated to 60 ºC. (3-Bromopropoxy)(*tert*-butyl)dimethylsilane **(2)** (12.6 g, 50 mmol, 1.1 equiv) was added and the mixture stirred at 60 ºC for 16 hours. TLC analysis indicated complete consumption of **1**. The mixture was cooled to room temperature and water (300 mL) added. The mixture was extracted with ethyl acetate (2 x 100 mL). The organic layers were combined and washed with water (2 x 50 mL) and brine then dried over magnesium sulfate. Concentration followed by silica column purification using 5% ethyl actetate/hexanes afforded product **3** as a colorless oil (16.0 g, 90%). ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.63 (d, 2H, ArH; *J* = 7.5 Hz); 6.71 (d, 2 ArH, *J* = 7.5 Hz); 4.03 (t, 2, OCH_2, *J* = Hz); 3.80 (t, 2H, OCH_2, *J* = 4.5 Hz); 2.00 (pentet, 2H, -CH_2**CH_2**CH_2O-, *J* = 4.5 Hz); 0.89 (s, 9H, t-Bu); 0.05 (s, 6, $SiCH_3$).

Synthesis of (*E*)-(3-(4-(3,5-bis((2-methoxyethoxy)methoxy)styryl)phenoxy)propoxy)(*tert*-butyl)dimethylsilane (4): A 125-mL, three neck round bottom flask was charged with compound **3** (2.5 g, 6.4 mmol, 1 equiv), compound **11** (2.0 g, 6.4 mmol, 1 equiv), tri-*o*-tolylphosphine (292 mg, 0.96 mmol, 15 mol%) and anhydrous 1-methyl-2-pyrrolidinone (20 mL, 10 vol). The mixture was vacuum purged with nitrogen for three times. Palladium acetate (72 mg, 0.32 mmol, 5 mol%) and triethylamine (1.3 g, 12.8 mmol, 2 equiv) were added and the mixture vacuum purged for three times with nitrogen. The mixture was stirred at 100 ºC for 12 hours and TLC indicated the completion of the reaction. The reaction mixture was cooled to room temperature, diluted with water (60 mL) and extracted with ethyl acetate (2 x 50 mL). The combined organics were washed with brine (100 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude was purified by silica column using 10-30% ethyl acetate/hexanes as the eluent to afford product **4** as light brown oil (2.4 g, 65%). ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.49-6.62 (m, 8H, ArH, vinylic CH); 5.29 (s with imbedded multiplet, 5H, -OCH_2O-; vinylic CH); 4.03 (t, 4H, OCH_2, *J* = 4.4 Hz); 3.85 (t, 4H, OCH_2, *J* = 4.4 Hz); 3.59 (s, 4H, CH_2); 3.41 (s, 6H, OCH_3) 2.00 (q, 2H, -CH_2**CH_2**CH_2O-, *J* = 4.4 Hz); 0.91 (s, 9H, t-Bu); 0.06 (s, 6, $SiCH_3$).

Preparation of (*E*)-3-(4-(3,5-bis((2-methoxyethoxy)methoxy)styryl)phenoxy)propan-1-ol (5): A 250-mL, three neck round bottom flask was charged with compound **4** (14.6 g, 25.4 mmol, 1 equiv) and anhydrous tetrahydrofuran (75 mL, 5 vol). TBAF (1M in THF, 38.2 mL, 38.2 mmol, 1.5 equiv) was added slowly and the mixture stirred at room temperature for 2 hours. TLC indicated the completion of the reaction. The reaction mixture was diluted with water (100 mL) and extracted with ethyl acetate (2 x 50 mL). The combined organics were washed with brine (100 mL), dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude was purified by silica column using 50-100% ethyl acetate/hexanes as the eluent to afford 11.2 g of the product, which is found to be a mixture of trans and cis-compound in 85:15 ratio. The product was re-purified by Teledyne ISCO system using a gold column (125 g cartridge) to afford 5.0 g of the pure product **5** as light brown clear oil (43%). ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.42 (d, 2H, ArH); 7.06-6.62 (multiplet, 7H, ArH; vinylic CH); 5.28 (s, 4H, -OCH_2); 4.15 (t, 2H, OCH_2, *J* = 4.5 Hz); 3.84 (overlapping multiplets, 6H, 2- CH_2; CH_2OH); 3.58 (t, 4H, 2 -CH_2, *J* = 4.5 Hz); 3.39 (s, 6H, -OCH_2); 2.13 (pentet, 2H, -CH_2, *J* = 4.5 Hz); 1.81 (br t, 1H, -OH).

Preparation of (*E*)-1-(4-(3-bromopropoxy)styryl)-3,5-bis((2-methoxyethoxy)methoxy) benzene (6): A 100-mL, three neck round bottom flask was charged with compound **5** (1.72 g, 3.72 mmol, 1 equiv), triphenylphosphine (1.27 g, 4.8 mmol, 1.3 equiv) and anhydrous dichloromethane (35 mL, 20 vol). Carbon tetrabromide (1.48 g, 4.46 mmol, 1.2 equiv) was added and the mixture stirred for 2 hours. TLC analysis indicated complete consumption of compound **5**. Water (40 mL) was added and the layers separated. The organic layer was dried over magnesium sulfate, filtered and concentrated. The crude product was purified by silica column (10-20% ethyl acetate/hexanes) to afford product **6** (1.6 g, 82%); ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.42 (overlapping d, 2H, ArH); 7.06-6.62 (multiplet, 7H, ArH; vinylic CH); 5.28 (s, 4H, -OCH_2); 4.12 (t, 2H, OCH_2, J = 4.5 Hz); 3.85 (t, 6H, CH_2, J = 4.8 Hz); 3.59 (overlapping multiplets, 4H, 2- CH_2; CH_2OH); 3.39 (s, 6H, -OCH_2); 2.05 (pentet, 2H, CH_2, J = 4.8 Hz).

Preparation of (S)-4-(3-(4-((E)-3,5-bis((2-methoxyethoxy)methoxy)styryl)phenoxy)-propoxy)-5-((R)-2,2-dimethyl-1,3-dioxolan-4-yl)-3-hydroxyfuran-2(5H)-one (8):
A 100-mL, three neck round bottom flask was charged with compound **6** (1 g, 1.9 mmol, 1 equiv), compound **7** (0.52 g, 2.38 mmol, 1.25 equiv) and anhydrous dimethyl sulfoxide (15 mL, 15 vol). Sodium bicarbonate (0.24 g, 2.86 mmol, 1.5 equiv) was added and the mixture stirred at 50 ºC for 16 hours. TLC analysis indicated complete consumption of compound **6**. The mixture was cooled to room temperature and water (60 mL) added. The mixture was extracted with ethyl acetate (2 × 50 mL). The combined organics were washed with water (30 mL), dried over magnesium sulfate, filtered and concentrated. The crude product was purified by Teledyne ISCO system using a gold column (40 g cartridge) using 20-60% ethyl acetate/hexanes as the eluent to afford the pure product **8** as light brown oil (30 mg; 2.4%); ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.42 (overlapping d, 2H, ArH); 7.06-6.62 (multiplet, 7H, ArH; vinylic CH); 5.93 (br s, 1H, OH); 5.28 (s, 4H -OCH_2); 4.70 (2 overlapping t, 2H, diastereotopic CH_2, J = 4.8 Hz); 4.55 (d, 1H, lactone CH); 4.24 (m, 1H); 4.10 (m, 4H, 2 x CH_2); 3.83 (t, 4H, CH_2, J = 4.8 Hz); 3.58 (t, 4H; CH_2, J = 4.8 Hz); 3.39 (s, 6H, 2 x CH_3); 2.23 (pentet, 2H, CH_2**CH_2**CH_2); 1.38 (s, 3H, CH_3); 1.34 (s, 3H, CH_3). HPLC shows 93.9% (AUC) purity.



Conclusion

The first known resveratrol-ascorbic acid conjugate was successfully prepared by a convergent synthesis in seven total steps. The lowest yielding step of the process was the coupling of the protected ascorbic acid with the bromoalkyl resveratrol derivative **6**. In addition to efforts to determine if this conjugate can pass the BBB in a rat brain homogenate assay, future work will include a study to optimize this coupling step.

References

1. Englard, S.; S. Seifter. (1986) The biochemical functions of ascorbic acid. *Ann. Rev. Nutr.* 6:365–406.
2. Padh, H. (1990) Cellular functions of ascorbic acid. *Biochem. Cell Biol.* 68: 1166–1173.
3. Kaufman, S. (1966) Coenzymes and hydroxylases: ascorbate and dopamine-β-hydroxylase; tetrahydropteridines and phenylalanine and tyrosine hydroxylases. *Pharmacol. Rev.* 18:61–69.

4. Agus, D.B.; Gambhir, S.S.; Pardridge W.M.; Spielholz, C.; José Baselga, J.; Juan Vera, J.C.; Golde, D.W. (1997) Vitamin C crosses the blood–brain barrier in the oxidized form through the glucose transporters. *J. Clin. Invest.* 100: 2842-2848.
5. Varoni, E.M; Lo Faro, A.F.; Sharifi-Rad, J.;Iriti, M. (2016) Anticancer mechanisms of resveratrol *Front Nutr.* 3; 8.
6. Rauf, A.; Imran, M.; Butt, M.S.; Nadeem, M.; Peters, D.G.; Mubarak, M.S. (2018) Resveratrol as an anti-cancer agent: a review *Crit Rev Food Sci Nutr*. 58: 1428-1447.
7. Monacelli, F.; Acquarone, E.; Giannotti, C.; Borghi, R.; Nencioni, A. (2017) Vitamin C, aging, and Alzheimer's Disease *Nutrients* 9:670.
8. Ma, T.; Tan M-S.; Yu, J-T.; Tan, L. (2014) Resveratrol as a therapeutic agent for Alzheimer's Disease *Biomed Res Int.* 2014; 2014: 350516.

Received on January 22, 2022.