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PHOTOLABILE 'CAGED' FATTY ACIDS CONTAINING A 1-(2'-NITROPHENYL)-1,2-ETHANEDIOL MOIETY

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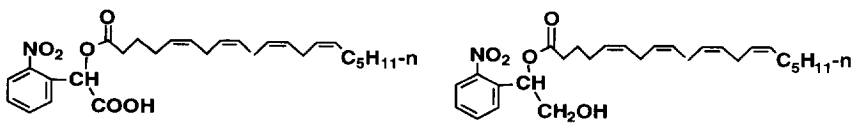
Abstract: 1-(2'-Nitrophenyl)ethanediol[1] was used to esterify the carboxylic acid function of fatty acids to prepare photosensitive fatty acid precursors for biological studies. The synthesis, photochemistry, and biological properties of several model *cis*-unsaturated fatty acids including arachidonic acid are described.

Photoactivated release of biochemical substrates has received considerable attention[2] since it was first reported that 2-nitrobenzyl phosphate esters of adenine nucleotides could efficiently release cyclic AMP or ATP upon photolysis.[3] Advantages are control of spatial and temporal distribution of a substrate within a complex system such as a living cell.[4] Fatty acids are important biochemical substrates and intracellular messengers that regulate many critical cell functions.[5] However, the development of photolabile fatty acid precursors has been slow in part because efficiencies for photochemical deprotection of carboxylic acids are generally poor. In addition, esterification of fatty acids reduces their solubility rendering them difficult to use in aqueous solutions. Introduction of the α-carboxyl-2-nitrobenzyl (CNB) caging group [6a] has led to many new caged molecules that are more biologically inert, more soluble in aqueous biological buffers, and photolyze with greater efficiency and speed than conventional 2-nitrobenzyl caged compounds.[6] The CNB moiety has also been attractive because of the possibility of further derivatizing the photosensitive group by esterification or amidation for incorporating labels, carriers or otherwise modifying the properties of caged molecules.

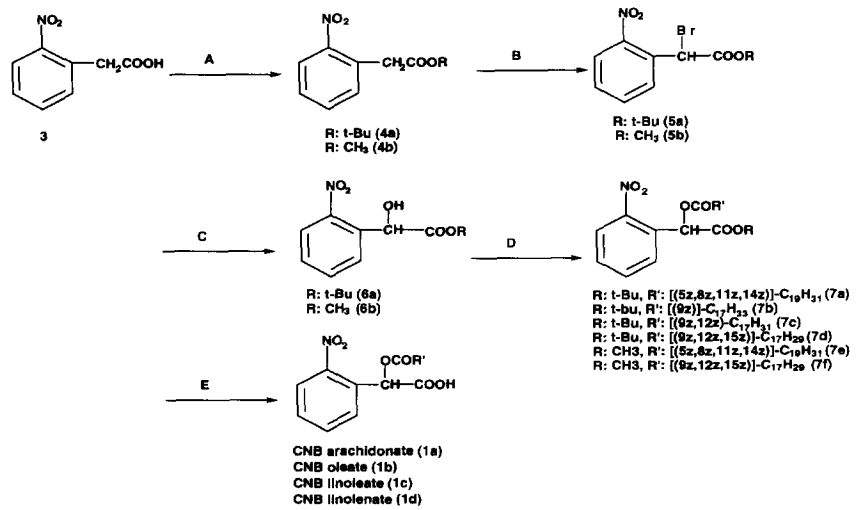
We report here that CNB fatty acids (Scheme 1) are chemically unstable and therefore largely unsuitable for use in biological studies. A new photosensitive protecting group for caboxylates, 1-(2'-nitrophenyl)-1,2 enthandiol (NED) (Scheme 1), is described that offers better stability than the CNB group, and retains good photolysis efficiency and aqueous solubility. We also provide evidence that these fatty acid derivatives will be useful for biological applications because they are easily incorporated into living cells, and are ineffective as activators of protein kinase C until after photolysis.



CNB arachidonate (1a) NED arachidonate (2a)

Scheme 1

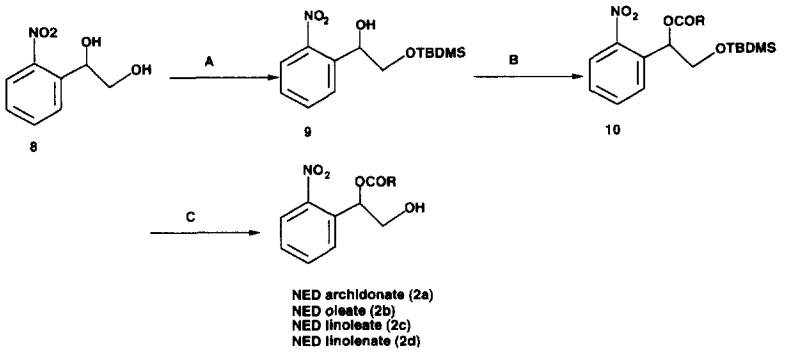
Synthesis of CNB Fatty Acid Esters



Scheme 2. (A) **4a**: $Cl_3CC(:NH)OBu$-t-$BF_3.Et_2O/CH_2Cl_2$, rt, 4-12 h, Yield: 85-90%. **4b**: MeOH-conc. H_2SO_4, refluxing, 48 h, Yield: 80-85%. (B) NBS/$(PhCOO)_2$-CCl_4, refluxing, 48 h, Yield: 40-60%. (C) $AgClO_4/CH_3COCH_3$-H_2O(2:1), rt, 3-4 days, Yield: 50-60%. (D) RCOOH/DCC-DMAP/CH_2Cl_2, rt, 6-12 h, Yield: 70-80%. (E) CF_3COOH, 0-5 °C, 3-4 h, Yield: 80-85%.

The synthesis of compounds **1a-d** was accomplished in five steps as illustrated in Scheme 2. 2-(2'-Nitrophenyl)acetic acid was first protected by esterification with t-butyl trichloroacetimidate in the presence of a catalytic amount of boron trifluoride diethyl etherate.[7] The resulting t-butyl 2-(2'-nitrophenyl)acetate **4a** was then brominated with N-bromosuccinimide (NBS) and catalytic benzoyl peroxide to yield compound **5a**. The bromide was converted to the corresponding alcohol, compound **6a**, by treatment with a $AgClO_4$-CH_3COCH_3-H_2O mixture.[8] Coupling of compound **6a** to the free fatty acids was accomplished by addition of dicyclohexylcarbodiimide (DCC), and 4-(N,N'-dimethylamino)pyridine (DMAP) as a catalyst. Finally, compounds **7a-d** were converted to CNB fatty acids **1a-d** by treatment with trifluoroacetic acid. By HPLC or TLC analysis, these CNB fatty acids **1a-d** degraded to uncharacterized products within several hours on ice whether kept as a dry solid or dissolved in ethanol, water, or 20 mM Tris pH 7.0. To avoid exposure of the compounds to strong acid during synthesis, we used a similar synthetic route but started with methyl 2-(2'-nitrophenyl)acetate **4b**, then removed the methyl protecting group in the final step with LiI in pyridine. The CNB fatty acids so prepared had the same properties described above, and because of this instability CNB fatty acids were not investigated further.

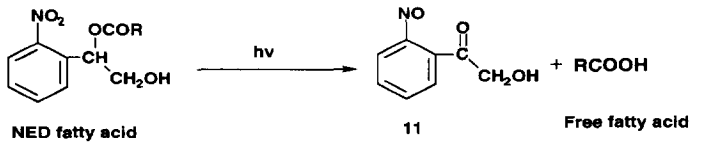
Synthesis of NED Fatty Acid Esters



Scheme 3. (A) TBDMSCl/DMAP-Et_3N/CH_2Cl_2, rt, 2-3 h, Yield: 80-90%. (B) RCOOH/DCC-DMAP/CH_2Cl_2, rt, 12 h, Yield: 80-85%. (C) 5 M HCl/MeOH-CH_2Cl_2, 0 ^{0}C, 3-4 h, Yield: 85-90%.

The synthesis of compounds **2a-d** was accomplished in three steps as illustrated in Scheme 3. The primary hydroxyl group of 1-(2'-nitrophenyl)-1,2-ethanediol (Aldrich Chemical Co) was first selectively protected by silylation with t-butyldimethylchlorosilane (TBDMSCl). Esterification of 1-(2'-nitrophenyl)-2-t-butyldimethylsilyl-1, 2-ethanediol **9** with a series of unsaturated fatty acids afforded 1-(2'-nitrophenyl)-1-acyl-2-t-butyldimethylsilyl-1,2-ethanediols **10** in good yield. Removal of the t-butyldimethylsilyl group was carried out in aqueous 5 M HCl, methanol and dichloromethane at 0 ^{0}C, similar to conditions previously developed to minimize acyl chain migration.[9] The lack of migration of the acyl chain during or after deprotection was confirmed by TLC, HPLC, NMR and by showing that compounds **2a-d** photolyzed to stoichiometric amounts of fatty acids.

Photochemistry and Biological Properties



Scheme 4. Proposed photolysis reaction for NED fatty acids

The predicted reaction for photochemical cleavage of the NED fatty acid esters is illustrated in Scheme 4. NED fatty acid esters in 20 mM Tris buffer pH 7 or in ethanol were exposed to a continuous 75 watt xenon lamp filtered

to pass 300-400 nm light. HPLC analysis of the photolysis mixture at different times revealed quantum yields of 0.18 (versus caged phenylephrine as internal standard).[6b] The main photolysis by-product was isolated from photolysis mixtures by HPLC and was shown by ^{1}H NMR to be consistent with compound **11**. Flash photolysis of compound **2b** in methanol revealed a transient absorbance signal at 420 nm consistent with a photolysis mechanism involving an *aci* -nitro intermediate[2] that decayed to products at 7 s^{-1} (0.01 N KOH) and 63 s^{-1} (10^{-4} N KOH) at 23 °C. Under more physiological conditions of 20 mM Tris pH 7.5 in aqueous solution, the absorbance transient was not observed presumably because it was too fast to measure (>1000 s^{-1}) with our instrument.

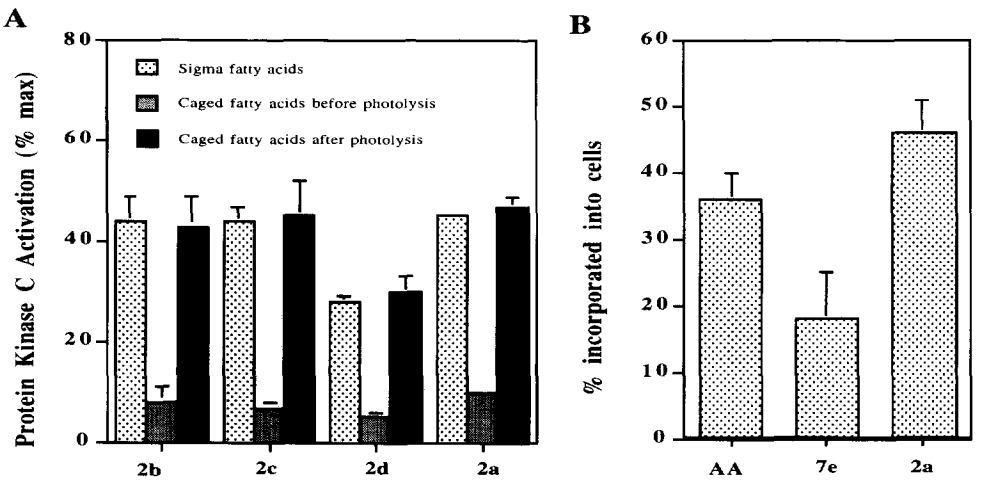


Figure 1. Biological properties of NED fatty acids. (**A**) Activation of protein kinase C by NED fatty acids before and after photolysis. (**B**) Incorporation of radiolabeled arachidonic acid (**AA**), NED arachidonate (**2a**) , methyl CNB arachidonate (**7e**) into cardiac cells.

Exhaustively photolyzed (10 min) NED fatty acids activated protein kinase C in vitro displaying similar potency to fatty acid standards (Figure 1A). Before photolysis, NED fatty acids displayed little or no capability to activate protein kinase C (Figure 1A). Incorporation of NED unsaturated fatty acids into cells dispersed in aqueous suspension was monitored by scintillation counting of ^{3}H-labeled compounds. Cardiac cells isolated from rat hearts were incubated at 37 °C with NED ^{3}H-fatty acids for various times and then centrifuged. The cell pellet was washed twice with Ringers solution[10] and counted. NED arachidonate **2a** was readily taken up by cardiac cells, with 47% incorporation of counts at equilibrium (10 min incubation) (Figure 1B). This level of incorporation was significantly greater than incorporation of AA itself or methyl CNB arachidonate **7e** (Figure 1B) suggesting that the free hydroxyl and possibly the aromatic ring on NED fatty acid esters facilitated intercalation into cell membranes.

In conclusion, CNB fatty acids were found to be unstable and therefore could not be reliably characterized or used as biological probes. The 1-(2'-nitrophenyl)2-ethanediol moiety, termed NED, may be a useful alternative photolabile protecting group for carboxylates in fatty acids and other biological molecules. Its advantages include chemical stability, clean photochemistry, and ease of use with biological samples in aqueous solution. NED also offers the versatility of a reactive alcohol side chain for linking these and other photolabile precursors to labels, beads or peptide sequences for targeting to specific cellular locations.

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References and Notes:

1. Hebert, J.; Gravel, D. *Can. J. Chem.* **1974**, 52, 187
2. (a) Gee, K. R.; Kueper, L. W. (III); Barnes, J.; Dudley, G.; Givens, R. S. *J. Org. Chem.* **1996**, 61, 1228. (b) Corrie, J. E. T.; Trentham, D. R. In Biological Applications of Photochemical Switches; Morrison , H. ed.; J. Wiley and Sons: New York, 1994, pp 243-305. (c) Walker, J. W.; Lu, Z.; Moss, R. L. *J. Biol. Chem.* **1992**, 267, 2459. (d) Walker, J.W.; Reid, G. P.; McCray, J. A.; Trentham, D. R. *J. Am. Chem. Soc.* **1988**, 110, 7170. (e) Walker, J. W.; Reid, G. P.; Trentham, D. R. *Methods Enzym.*ol **1989**, 172, 288.
3. (a) Engels, J.; Schlaeger, E-J. *J. Med. Chem.* **1977**, 20, 907. (b) Kaplan, J. H.; Forbush, B. III; Hoffman, J. F. *Biochemistry*, **1978**, 17, 1929.
4. Sreekumar, R.; Pi, Y. Q.; Huang, X. P.; Walker, J. W. *Bioorg. Med. Chem. Lett.* **1997**, 7, 341.
5. (a) Kang, J. X.; Leaf, A. Proc. Natl. Acad. Sci. U.S. A. **1994**, 91, 9886. (b) Kang, J. X.; Xiao, Y-F.; Leaf, A. *Proc. Natl. Acad. Sci. U.S. A.* **1995**, 91, 3997.
6. (a) Milburn, T.; Matsubara, N.; Billington, A. P.; Udgaonkar, J.B.; Walker, J. W.; Carpenter, B.K.; Webb, W. W.; Marque, J.; Denk, W.; McCray, J. A.; Hess, G. P. *Biochemistry* **1989**, 28, 49. (b) Walker, J. W. Martin, H.; Schmitt, F. R.; Barsotti, R. J. *Biochemistry*, **1993**, 32, 1338.
7. Armstrong, A.; Brackenridge, I.; Jackson, R. F. W.; Kirk, J. M. *Tetrahedron Lett.* **1988**, 29, 2483.
8. Kashman, Y.; Edwards, J. A. *J. Org. Chem.* **1978**, 43, 1538.
9. (a) Morgan, D.; Ramesgha, C.; Romero, M.; Talamas, F. *Bioorg. Med. Chem. Lett.* **1994**, 4, 827. (b) Xia, J.; Hui, Y-Z, *Bioorg. Med. Chem. Lett.* **1995**, 5, 2815. (c) Starck, J-P.; Nakatani Y.; Ourisson, G. *Tetrahedron* **1995**, 51, 2629.
10. Huang, X. P.; Sreekumar, R.; Patel, J. R.; Walker, J. W. *Biophys, J.* **1996**, 70, 2448.
11. **1a:** ^{1}H NMR ($CDCl_3$): δ 8.20-8.10 (d, 1H, Ar**H**), 7.90-7.30 (m, 3H, Ar**H**), 6.60-6.40 (s, 1H, C**H**OCOR), 5.50-5.30 (m, 8H, 4C**H**=C**H**), 2.90-2.70 (t, 6H, 3=CC**H**$_2$C=), 2.40-2.30 (t, 2H, C**H**$_2$CO), 2.10-1.90 (m, 4H, 2C**H**$_2$C=), 1.70-1.50 (m, 2H), 1.40-1.20 (s, 6H), 0.90-0.80 (t, 3H, C**H**$_3$). **1b:** ^{1}H NMR ($CDCl_3$): δ 8.00-7.75 (d, 1H, Ar**H**), 7.75-7.30 (m, 3H, Ar**H**), 6.50-6.30 (s, 1H, C**H**OCOR), 5.50- 5.30 (m, 2H, C**H**=C**H**), 2.50-2.30 (t, 2H, C**H**$_2$CO), 2.10-1.90 (m, 4H, 2C**H**$_2$C=), 1.70-1.20 (m, 22H), 1.00-0.90 (t, 3H, C**H**$_3$). **1c:** ^{1}H NMR ($CDCl_3$): δ 8.00-7.80 (d, 1H, Ar**H**), 7.80-7.30 (m, 3H, Ar**H**), 6.75-6.50 (s, 1H, C**H**OCOR), 5.50-5.25 (m, 4H, 2C**H**=C**H**), 3.00-2.80 (t, 2H, =CC**H**$_2$C=), 2.50-2.30 (t, 2H, C**H**$_2$CO), 2.00-1.90 (m, 4H, 2C**H**$_2$C=), 1.70-1.20 (m, 16H), 0.90-0.80 (t, 3H, C**H**$_3$). **1d:** FABMS (m/e): 457 (M^+). ^{1}H NMR ($CDCl_3$): δ 8.00-7.90 (d, 1H, Ar**H**), 7.70-7.60 (m, 1H, Ar**H**), 7.60-7.50 (m, 1H, Ar**H**), 7.50-7.40 (m, 1H, Ar**H**), 6.50-6.40 (s, 1H, C**H**OCOR), 5.50-5.40 (m, 6H, 3C**H**=C**H**), 2.90-2.80 (t, 4H, 2=CC**H**$_2$C=), 2.40-2.30 (t, 2H, C**H**$_2$CO), 2.10-

2.00 (m, 4H, 2C**H**$_2$C=), 1.70-1.50 (m, 2H), 1.50-1.20 (s, 8H), 1.00-0.80 (t, 3H, C**H**$_3$). **7b:** EIMS (m/e): 518 (M^++1), 461 (M^+-C_4H_9), 265 ($C_{17}H_{33}CO^+$), 79 (100%). ^{1}H NMR ($CDCl_3$): δ 8.00-7.90 (d, 1H, Ar**H**), 7.70-7.60 (d, 3H, Ar**H**), 6.70-6.60 (s, 1H, C**H**OCOR), 5.40-5.30 (t, 2H, C**H**=C**H**), 2.50-2.40 (t, 2H, C**H**$_2$CO), 2.00-1.90 (m, 4H, 2C**H**$_2$C=), 1.70-1.60 (m, 2H), 1.40-1.10 (s, 29H), 0.90-0.80 (t, 3H, C**H**$_3$). **7e:** ^{1}H NMR ($CDCl_3$): δ 8.10-8.00 (d, 1H, Ar**H**), 7.65-7.50 (m, 3H, Ar**H**), 6.90-6.80 (s, 1H, C**H**OCOR), 5.50-5.30 (m, 8H, 4C**H**=C**H**), 3.80-3.70 (s, 3H, OC**H**$_3$), 2.90-2.70 (t, 6H, 3=CC**H**$_2$C=), 2.40-2.30 (t, 2H, C**H**$_2$CO), 2.10-2.00 (m, 4H, 2C**H**$_2$C=), 1.70-1.60 (m, 2H), 1.40-1.20 (s, 6H), 0.80-0.70 (t, 3H, C**H**$_3$). **7f:** ^{1}H NMR ($CDCl_3$): δ 8.10-8.00 (dd, 1H, Ar**H**), 7.70-7.50 (m, 3H, Ar**H**), 6.90-6.80 (s, 1H, C**H**OCOR), 5.50-5.30 (m, 6H, 3C**H**=C**H**), 3.80-3.70 (s, 3H, OC**H**$_3$), 2.90-2.80 (t, 4H, 2=CC**H**$_2$C=), 2.50-2.30 (t, 2H, C**H**$_2$CO), 2.10-2.00 (m, 4H, 2C**H**$_2$C=), 1.70-1.50 (m, 2H), 1.40-1.20 (s, 8H), 1.00-0.80 (t, 3H, C**H**$_3$). **2a:** FABMS (m/e): 470 (M^++1), 303 ($C_{19}H_{31}CO^+$), 166 ($NO_2PhCHCH_2OH^+$) (100%). ^{1}H NMR ($CDCl_3$): δ 8.10-8.00 (d, 1H, Ar**H**), 7.70-7.60 (m, 2H, Ar**H**), 7.50-7.4 (m, 1H, Ar**H**), 6.50-6.40 (dd, 1H, C**H**OCO), 5.50-5.40 (m, 8H, 4C**H**=C**H**), 4.20-3.90 (m, 2H, C**H**$_2$OH), 2.90-2.80 (m, 6H, 3 =CC**H**$_2$C=), 2.50-2.40 (t, 2H, C**H**$_2$CO), 2.20-2.00 (m, 4H, 2C**H**$_2$C=), 1.70-1.60 (t, 2H), 1.40-1.30 (m, 6H), 0.90-0.80 (t, 3H, C**H**$_3$). **2b:** FABMS (m/e): 447 (M^+), 265 ($C_{17}H_{33}CO^+$), 166 ($NO_2PhCHCH_2OH^+$) (100%). ^{1}H NMR ($CDCl_3$): δ 8.10-8.00 (d, 1H, Ar**H**), 7.70-7.60 (m, 2H, Ar**H**), 7.50-7.40 (m, 1H, Ar**H**), 6.50-6.40 (dd, 1H, C**H**OCO), 5.40-5.30 (m, 2H, C**H**=C**H**), 4.20-3.90 (m, 2H, C**H**$_2$OH), 2.50-2.40 (t, 2H, C**H**$_2$CO), 2.10-2.00 (m, 4H, 2C**H**$_2$C=), 1.70-1.50(m, 2H), 1.40-1.30 (s, 20H), 0.90-0.80 (t, 3H, C**H**$_3$). **2c:** FABMS (m/e): 468 (M^++1+Na), 444 (M^+-1), 263 ($C_{17}H_{31}CO^+$), 166 ($NO_2PhCHCH_2OH^+$) (100%). ^{1}H NMR ($CDCl_3$): δ 8.00-7.90 (d, 1H, Ar**H**), 7.60-7.50 (m, 2H, Ar**H**), 7.50-7.40 (m, 1H, Ar**H**), 6.40-6.30 (dd, 1H, C**H**OCO), 5.50-5.30 (m, 4H, 2C**H**=C**H**), 4.10-3.90 (m, 2H, C**H**$_2$OH), 2.90-2.80 (t, 2H, =CC**H**$_2$C=), 2.50-2.40 (t, 2H, C**H**$_2$CO), 2.10-2.00 (m, 4H, 2C**H**$_2$C=), 1.70-1.50 (t, 2H), 1.40-1.30 (s, 14H), 0.90-0.80 (t, 3H, C**H**$_3$). **2d:** FABMS (m/e): 444 (M^+ +1), 261 ($C_{17}H_{29}CO^+$), 166 ($NO_2PhCHCH_2OH^+$) (100%). ^{1}H NMR ($CDCl_3$): δ 8.00-7.90 (d, 1H, Ar**H**), 7.70-7.40 (m, 3H, Ar**H**), 6.50-6.30 (dd, 1H, C**H**OCO), 5.50-5.20 (m, 6H, 3C**H**=C**H**), 4.20-3.90 (m, 2H, C**H**$_2$OH), 2.80-2.70 (t, 4H, 2=CC**H**$_2$C=), 2.50-2.40 (t, 2H, C**H**$_2$CO), 2.10-1.90 (m, 4H, 2C**H**$_2$C=), 1.70-1.50 (t, 2H), 1.40-1.30 (s, 8H), 1.05-0.80 (t, 3H, C**H**$_3$). **11**. ^{1}H NMR ($CDCl_3$): δ 8.20-8.10 (d, 1H, Ar**H**), 7.80-7.65 (dd, 2H, Ar**H**), 7.50 (t, 1H, Ar**H**), 5.50 (d, 2H, C**H**$_2$OH), 2.70-2.60 (t, 1H, O**H**).

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