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Synthesis of Photosensitive EGTA Derivatives

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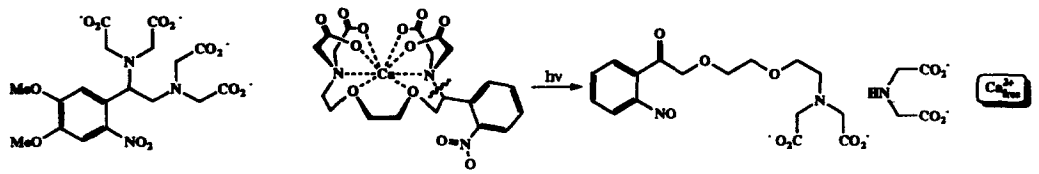
Summary. The syntheses of five derivatives of EGTA and one EGTA analogue are described. These molecules each have an *ortho*-nitrophenyl substituent positioned such that irradiation will cut the EGTA coordination sphere in two. © 1998 Elsevier Science Ltd. All rights reserved.

The past 20 years have seen the concomitant development of two photochemical techniques for studying living cells: rapid concentration jumps of cellular effectors from biologically inert precursors (caged compounds[1]) and imaging the concentration changes of small solutes using light microscopy.[2] This paper concerns the synthesis of photolabile derivatives of EGTA, a Ca^{2+}-selective chelator, designed so that concentration jumps in intracellular Ca^{2+} can be achieved by irradiation with near-u.v. light (320-400 nm).

Cells use many means to transmit signals received by receptors on their surface to create second messengers in the cytoplasm. The most common intracellular signal transduction agent is Ca^{2+}, which, unlike other second messengers, is not metabolised by enzymes resident within the cell.[3] Ca^{2+} sequestration/ extrusion mechanisms are present through which cells tightly regulate intracellular Ca^{2+} concentration at a level which is subthreshold or non-activating for the myriad of Ca^{2+}-dependent physiological processes.[4] Examples of such processes are muscle contraction,[5] neurotransmission,[6] ion channel gating,[7] gene expression,[8] secretion,[9] cell division,[10] etc.

The strategy developed in this laboratory during the past 10 years for the release of Ca^{2+} uses the *o*-nitrobenzyl protecting group, which was discovered in 1966 by Baltrop et al.[11] and the utility of which was greatly extended by Woodward and co-workers.[12] This caged Ca^{2+} protocol involves the synthesis[13,14] of photolabile (*o*-nitrophenyl) derivatives of molecules of known high affinity for Ca^{2+}; illumination cuts the chelator in two, producing molecules of known low affinity, rapidly[15] releasing the bound Ca^{2+} (*vide infra*). Two such Ca^{2+} cages are commercially available: DM-nitrophen[16] and NP-EGTA.[14]



DM-nitrophen NP-EGTA-Ca^{2+} photolysis

This paper describes the synthesis of a range of photolabile Ca^{2+}-selective chelators which are designed to combine the optical properties of the former with the cation selectivity of the later. *Six new Ca^{2+} cages have been synthesized 1-6, as outlined in Schemes 1, 2 & 3.*

The construction of the ethyleneglycol backbone of EGTA is the key synthetic challenge for the development of photolabile derivatives of this Ca^{2+}-specific chelator.[13] Three different strategies were employed, as shown in Schemes 1, 2 and 3. In the synthesis of dimethoxy derivatives of NP-EGTA (**1-3**),

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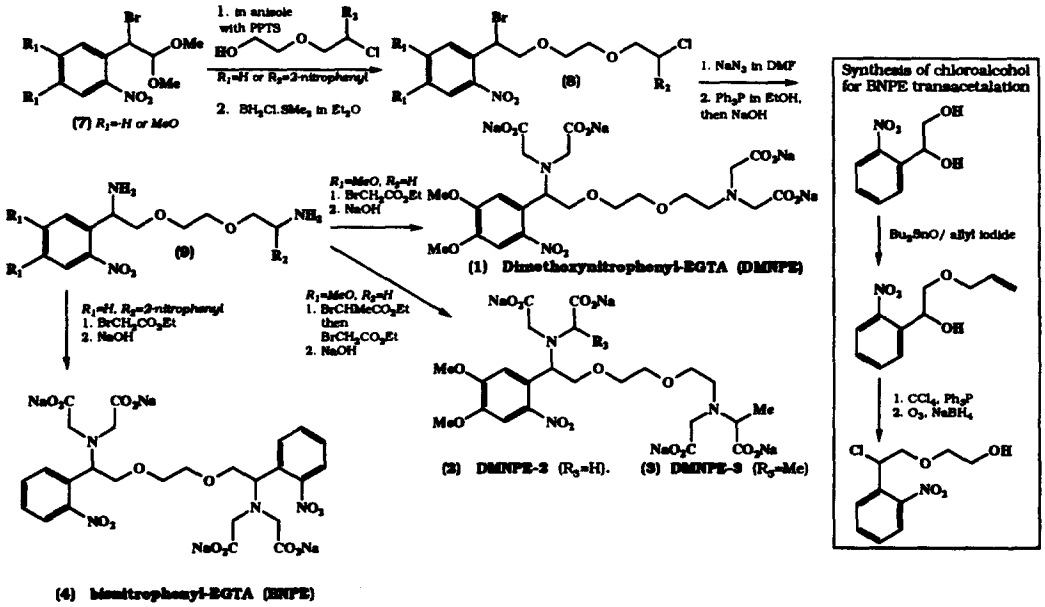
modification of the original procedure[14] proved very effective. The key chemical transformations proved to be transacetalation of the readily available[14] bromodimethoxy acetal **7** with 2-(2-chloroethoxy)ethanol, followed by reduction of the acetal to give a molecule having the ethylene glycol backbone of an EGTA-like chelator (i.e. **8**), bearing the requisite functionality for elaboration into an array of Ca^{2+} cages (Scheme 1).

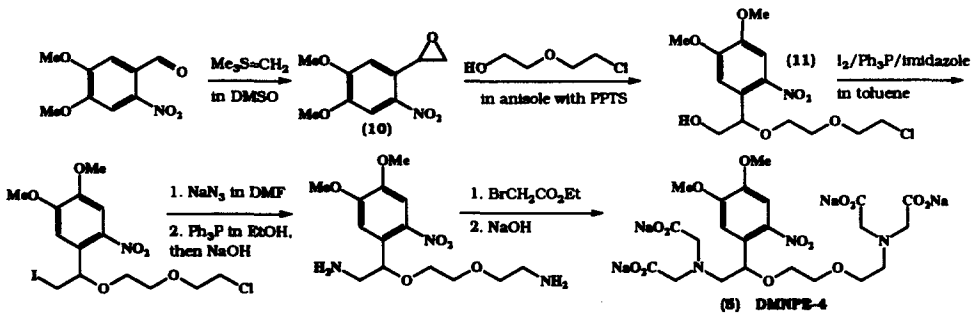


SCHEME 1

Effective construction of this crucial part of the Ca^{2+} coordination sphere by this route proved to be very dependent upon solvent, concentration of the reactants, and the acid catalyst employed in the first of the two steps. A wide range of conditions were examined and by far the most effective catalyst proved to be PPTS, with anisole as the solvent. Coupling was realised when the concentration of bromide **7** was 0.25 M and the chloroalcohol was 2.0 M; the reaction mixture was heated for 7 h. at 100°C. Yields for the two-step conversion of **7** to **8** were 61% (R_1=MeO, R_2=H) and 33% (R_1=H, R_2=*o*-nitrophenyl). Dihalide **8** was then converted in to diamine **9** (71 % yield) via reduction of the diazide.[14] Tetraalkylation of **9** with ethyl bromoacetate, followed by ester hydrolysis produced **1** (dimethoxynitrophenyl-EGTA, DMNPE) and **4** (bisnitrophenyl-EGTA, BNPE) in 61% and 43% yields respectively. Alkylation of diamine **9** (R_1=MeO, R_2=H) with isopropyl bromoacetate (10 mole equivalents in MeCN, reflux, 22 h.) gave mono and dialkylated products (in yields of 22 and 43% respectively; due to steric hindrance, the benzylic amine is much less reactive than the amine distal to the chromophore. No trialkylation was observed). The syntheses were completed by exhaustive alkylation of these (purified) amines with ethyl bromoacetate, followed by ester hydrolysis, to give DMNPE-2 (**2**) and DMNPE-3 (**3**).

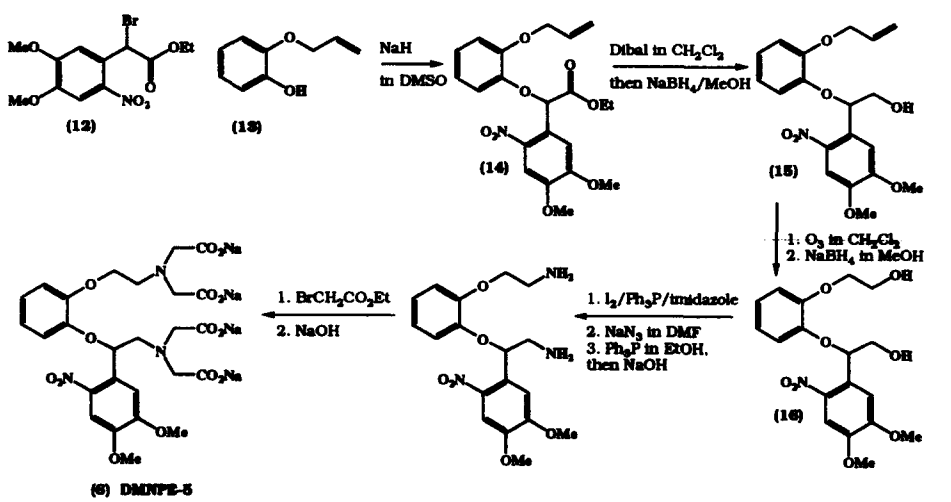
An alternative synthesis of a photosensitive ethyleneglycol backbone of EGTA was accomplished by regioselective alkylation of the readily available epoxide **10** with 2-(2-chloro-ethoxy)ethanol. The most suitable conditions for effective coupling (47% yield) proved to be similar to the transacetalation above, namely PPTS catalyst in anisole, at high concentrations of reactants (0.2 M epoxide, 1 M alcohol), heating

the reaction mixture for 2 h. at 80°C. Using other catalysts produced rapid decomposition of the epoxide, as did lower reactant concentrations: epoxide self-condensation probably competes very effectively with ring opening by the chloroalcohol. Transformation of **11** into the target Ca^{2+} cage **5** proceeded without incident. Iodination was effected[13] smoothly (95%), followed by conversion of the diiodide to DMNPE-4 in 45% overall yield for the remaining four steps (Scheme 2).



SCHEME 2

The final synthetic route to a photosensitive EGTA-like molecule is outlined in Scheme 3. This route was designed in the hope that a much more chemically efficient route to a Ca^{2+}-specific chelator might be developed, since alkylation of phenols proceeds under much milder conditions than regular alcohols to produce ether linkages. Addition of bromoester **12** (the precursor to **7**, R_1=MeO) to a solution of the anion of the readily available phenol **13** (from the monoalkylation of pyrocatechol with allyl bromide) in DMSO yielded **14** in a 60% yield (the reaction was complete upon addition of the bromide at RT). Reduction of ester **14** with Dibal in CH_2Cl_2 (completed by addition of $NaBH_4$ in MeOH) to alcohol **15** was facile (92%), which was converted to diol **16** by in situ reduction of the ozonide (55%). Elaboration of this diol into the final product **6** was accomplished by the same synthetic sequence employed for the other chelators (*vide supra*), in an overall yield of 58%.



SCHEME 3

In conclusion, the syntheses outlined in Scheme 1 demonstrate that the previously developed[14] synthetic route for NP-EGTA is of a more general scope. Furthermore, two new synthetic routes for the construction of photosensitive EGTA derivatives have been established. That shown in Scheme 2 is accomplished in seven synthetic steps with an overall yield of 13%. Whereas the synthesis of EGTA analogue **6** requires 11 steps in all but is slightly more chemically efficient (16% yield). Preliminary data indicates that DMNPE-4 is the most promising new Ca^{2+} cage described herein, since it releases Ca^{2+} with a similar time-course to NP-EGTA[15] but has a larger molar absorbtivity (4,600 M^{-1} cm^{-1} vs. 975 M^{-1} cm^{-1}). Therefore, it has proved to be much more efficient in releasing Ca^{2+} in secretory cells than any Ca^{2+} cage thus far developed.[17]

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References.

1. Kaplan, J.H.; Somlyo, A.P. *Trends Neuro. Sci.* **1989**, *12*, 54-59; Corrie, J.E.T.; Trentham, D.R. In *Bioorganic Photochemistry 2* Morrison, H. Ed.; John Wiley & Sons, Inc.: New York, 1993; pp. 243-303; Adams, S.R.; Tsien, R.Y. *Annu. Rev. Physiol.* **1993**, *55*, 755-784; Givens, R.S.; Keuper, L.W. *Chem. Rev.* **1993**, *93*, 55-66. Zucker, R.S. *Meth. Cell Biol.* **1994**, *40*, 31-63; Nerbonne, J.M. *Curr. Op. Neurobiol.* **1996**, *6*, 379-386.

2. Tsien, R.Y. *Annu. Rev. Neurosci.* **1989**, 12, 227-253; *The Handbook of Confocal Microscopy* Pawley, J. Ed.; Plenum Press, New York, 1995.

3. Berridge, M.J. *Nature* **1993**, 361, 315-325; Clampham, D.E. *Cell* **1995**, 80, 259-268.

4. Bers, D.M. *Excitation-Contraction Coupling and Cardiac Contractile Force* Kluwer, Dordrecht, 1991.

5. Ebashi, S.; Endo, M.; Ohtsuki, I. *Q. Rev. Biophys.* **1969**, *2*, 351-384.

6. Augustine, G.J.; Charlton, M.P.; Smith, S.J. *Annu. Rev. Neurosci.* **1987**, *10*, 633-6937.

7. Györke, S.; Fill, M. *Science* **1993**, *260*, 807-809.

8. Brostom, C.O.; Brostom, M.A. *Annu. Rev. Physiol.* **1991**, *52*, 577-590; Gallin, W.J.: Greenberg, M.E. *Curr. Op. Neurobiol.* **1995**, *5*, 367-374; Ginty, D.D. *Neuron* **1997**, *18*, 183-186.

9. Neher, E. *Biochem. Soc. Trans.* **1993**, *21*, 420-423; Henkel, A.W.; Almers, W. *Curr. Op. Neurobiol.* **1996**, *6*, 350-357.

10. Whitaker, M.J. *Adv. Second Mess. Phosphoprot. Res.* **1995**, *108*, 525-542.

11. Baltrop, J.A.; Plant, P.J.; Schofield, P. *J. Chem. Soc., Chem. Commun.* **1966**, 822-823.

12. Patchornik, A.; Amit, B.; Woodward, R.B. *J. Am. Chem. Soc.* **1970**, *92*, 6333-6335; for reviews see Amit, B.; Zehavi, U.; Patchornik, A. *Isreal J. Chem.* **1974**, *12*, 103-113; Binkley, R.W.; Flechtner, T.W. in *Synthetic Organic Photochemsitry* Horspool, W.H., Ed.; Plenum: New York, 1984; pp. 375-423.

13. Ellis-Davies, G.C.R.; J.H. Kaplan. *J. Org. Chem.* **1988**, *53*, 1966-1969.

14. Ellis-Davies, G.C.R.; J.H. Kaplan. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 187-191.

15. Ellis-Davies, G.C.R.; Kaplan, J.H.; Barsotti, R.J. *Biophys. J.* **1996**, *70*, 1006-1016.

16. Kaplan, J.H.; Ellis-Davies, G.C.R. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 6571-6575.

17. Parsons, T.D.; Ellis-Davies, G.C.R.; Almers, W. *Cell Calcium* **1996**, *19*, 185-192, in this study we show that using a caged Ca^{2+} with dimethoxy substituents is essential to release enough Ca^{2+} to saturate the low affinity receptor which controls secretion. For preliminary studies using DMNPE-4 see: Sankaranarayanan, S.; Ellis-Davies, G.C.R.; Almers, W. *Soc. Neurosci. Abtr.* **1997**, *23*, 1172.