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Protocol for making 500 mg of photo-beads

By Huilin Zhou, updated 7/29/03

1) 500 mg of amino glass beads (Sigma, G4518) should have 400 µmol/g free amino groups. Load in an empty Bio-Rad column (cat 731-1550) or whatever suitable size column. Wash once by one column volume of anhydrous DMF (Aldrich).
2) **Link**: Dissolve 600 µmol each of HoBt (MW=153, in fridge, Nova Biochem, 01-62-0008) and Fmoc-aminoethyl photolinker (MW=520, in drawer, Nova Biochem, 01-60-0042) in 4 ml of dry DMF completely. Add to this solution 600 µmol of diisopropylcarbodiimide (MW=126, d=0.8, in desiccator, Aldrich, D12540-7) for 30min. (Anytime when light-sensitive reagent is used, protect from light.)
3) Add 2) to the beads in 1) for 90 min incubation.
4) Wash beads by 3 column volumes of DMF and 2 column volumes of dry dichloromethane (Aldrich). Always remove excess solvent.
5) **Block**: Prepare a 2 ml mixture of 20% acetic anhydride (in desiccator, Aldrich), 30% pyridine (in hood), and 50% dichloromethane (hood). Add this mixture to the beads for 30min.
6) Wash beads by 2 column volumes of dichloromethane and 3 column volumes of DMF. Remove excess DMF.
7) **De-protect**: Make 4 ml of 20% piperidine (hood) in DMF (All common organics are from Aldrich). Incubate beads in *2 ml* of 20% piperidine/DMF for 30 min. Reserve 2 ml for blank. Collect all of the 2 ml flowthrough. Use 20% piperidine/DMF as blank and measure A_{290} (absorbance at 290nm) of the 1/500 dilution (dilute by 20% piperdine/DMF) of the flowthrough. The A_{290} should be between 0.3 and 0.4. Record the value. Calculate capacity: (A_{290} x dilution factor x flowthrough volume (ml))/(1.65 x weight of beads (mg)) = capacity (µmol/g) (should be ~ 400)
8) Wash beads by 5 column volumes of dry DMF again. Now the beads are ready for the next round of attachment of amino acid.
9) Repeat step 2 to 8 with an isotope-tagged linker other than the Fmoc-photolinker. For example, Fmoc-d0-GABA (Nova Biochem, 04-12-1088) or Fmoc-d6-GABA (protocol below).
10) **Attach iodoacetyl group**: Dissolve 600 µmol of iodoacetic anhydride (MW=354, fridge, Aldrich, light sensitive) in suitable volume of dry DMF (about 4 ml) before adding to the beads. Immediately after, add in 660 µmol of diisopropyl ethyl amine (FW=129, d=0.74, dessicator, Aldrich, D12580-6) to the beads mixture and mix it well. Let it incubate for 90 min.
11) Wash the beads with 5 column volumes of DMF and excess methanol. Dry in covered Bio-Spin columns in the Speedvac. Now the d0/d6-GABA-photo-beads are ready to use.

Protocol for Fmoc-d6-GABA

(600 µmol for 500 mg beads) (Andy Tao's improved protocol)

1) Dissolve 600 µmol d6-GABA (MW=109, shelf, Isotec) in 3 ml 10% sodium carbonate in H_20, in glass vial with stir bar.
2) Dissolve 600 µmol Fmoc-Osu (MW=337, -20C, Nova Biochem) in 3 ml dimethoxyethane. Add drop-wise to d6-GABA in vial. Stir at least 2 hours.
3) Add 20 ml H_20, making clear solution. Centrifuge to remove precipitate.
4) Add up to 600 µl conc. HCl to pH ~2. White precipitate forms.

5) Extract Fmoc-GABA with 3-4 ml ethyl acetate; collect organic phase and retain aqueous to repeat extraction 4-5 times, combining extracts.
6) Wash extract with H_20.
7) Weigh 4 empty microtubes and record weights. Dry extract (about 15 ml) in microtubes, adding more to tubes as it dries. Record final weights. Compare to calculated weight of 600 µmol of d6-Fmoc-GABA (MW=331) = 199 mg.

Step	beads reagent	100 mg 120 µmol	500 mg 600 µmol
Attach photolinker	DMF HoBt Fmoc photo-linker DIC	800 µl 18.4 mg 62.4 mg 18.9 µl	4 ml 92 mg 312 mg 95 µl
Block	Dichloromethane Acetic anhydride Pyridine	500 µl 200 µl 300 µl	1 ml 400 µl 600 µl
De-protect	DMF Piperidine	1.6 ml 400 µl	3.2 ml 800 µl
Link GABA	DMF HoBt Fmoc-d0-GABA DIC	800 µl 18.4 mg 39 mg 18.9 µl	4 ml 92 mg 195 mg 95 µl
Attach iodo	DMF Iodoacetic anhydride Diisopropyl ethyl amine	800 µl 42.5 mg 23 µl (132 µmol)	4 ml 212 mg 115 µl (660 µmol)
Fmoc-GABA	Sodium carbonate/H_2O d6-GABA Fmoc-Osu/DMF		300 mg/3 ml 65 mg 202 mg/3 ml