Desymmetrization Reactions: Efficient Preparation of Unsymmetrically Substituted Linker Molecules

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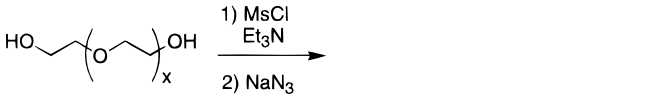
The utility of hydrophilic tethers for linkage of biomolecules without interference with their activity has become widely recognized to be important for affinity chromatography, combinatorial library presentation, and soluble reagent preparation. Among the most useful are the poly(ethylene glycol)-derived substances, because of their stability, polarity, and ready availability.[1] Unsymmetrically substituted derivatives have proven of particular use because sequential linkage of such derivatives to two different substances is straightforward.[2] Monoprotected diamines derived from tetraethylene glycol have proven useful as biocompatible spacer arms.[3,4] Amino azide **1a** represents a particularly useful example of such an unsymmetrical derivative, because the azide can be converted to the amine under several different mild reductive conditions to which biomolecules are resistant.[3] Bednarski's preparation of **1a** from tetraethylene glycol by statistical monomesylation, substitution by azide, chromatographic isolation of the monoazide, mesylation, phthalimide displacement, and hydrazinolysis proceeds in a remarkable 44% overall yield, considering the statistical process limits the maximal yield to 50%.

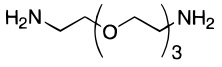
We found linker **1a** to be quite useful for our assay which depends on surface-tethered reagents to be enzyme-accessible.[5] We therefore sought a route to this substance that was more amenable to large-scale preparation:



2
2a; x = 3

1
1a; x = 3

We decided to investigate the reduction of diazide **2a** to amino azide **1a**, despite the careful study of reaction conditions sometimes necessary to make such desymmetrizations no worse than statistical.[6] Reduction of tetraethylene glycol diazide in methanol with $SnCl_2$ yields a statistical mixture of starting diazide **2a**, amino azide **1a**, and undesired diamine **3a**. This is not a satisfactory preparative procedure because of the difficulty of separation of **1a** from diamine **3a**.



3
3a; x = 3

Since the diazide is readily separable by extraction from the amines, one approach would be to carry the reaction to very low conversion, separate monoamine **1a** as it forms, and recycle diazide **2a**. We have found a simpler procedure that in effect accomplishes this. Reaction of **2a** with triphenylphosphine in ether leads cleanly to amino azide **1a**, as long as the ether layer is in vigorously stirred contact with dilute aqueous phosphoric acid. The acid protonates amine (and phosphinimine intermediate) as it forms, causing it to be extracted into the aqueous layer, thus sequestering it from contact with ether-soluble phosphine and preventing overreduction. This simple procedure is highly effective. Addition of base and extraction give amino azide **1a** substantially free of diamine **3a**. Treatment of the reaction mixture with base and then benzyl chloroformate and analyzing the derivatized monoamine and diamine by HPLC gave a ratio of amino azide **1a** to diamine **3a** of 200:1, confirming that the reaction, and not just the isolation, is selective.

Interestingly, the difficulty in preparative use of this reaction was in separation of the amino azide **1a** from triphenylphosphine oxide. Extensive extraction of the acidic aqueous solution of amino azide and phosphine oxide with various organic solvents removed only a small portion of the phosphine oxide. However, when the aqueous solution was made basic to allow extraction of the amino azide, the phosphine oxide was also extracted. Plausibly, protonated amine associates with phosphine oxide strongly enough to prevent its extraction from water. The mechanism and molecularity of this association remains to be investigated, but it is consistent with the very strong hydrogen-bonding ability of phosphine oxides,[7] as well as a hydrophobic interaction.[8]

Removal of triphenylphosphine oxide can be deferred; chromatographic separation of the phosphine oxide from amide derivatives of **1a** is straightforward.[5] Isolation of **1a** is accomplished by making the aqueous phase of the reaction mixture basic in the absence of organic solvent. Slow crystallization of the triphenylphosphine oxide takes place, and after its removal amino azide **1a** can be isolated in >80% yield and >98% purity by extraction. This preparation was scaled up to 20−50-g reactions. At

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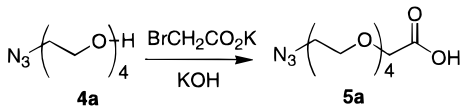
these scales, higher concentrations of reagents were used than in the previous cases, but high selectivity (>50:1) for monoreduction was still observed.

We were interested in exploring the scope of the selectivity, particularly the size of the molecule which can be selectively reduced. Poly(ethylene glycol)s 200, 300, and 400 were mixed and converted to diazides **2** via the mesylates. This mixture of diazides gave a mixture of amino azides under our selective reduction conditions. Thin-layer silica gel chromatography resolves mixed monoamines **1** from the diamines **3**.

We found that the high-concentration preparative conditions with ninhydrin quantitation[9] gave ratios of amino azide **1a** to diamine **3a** of 66:1. Under similar conditions the mixture of chain lengths **2** gave a similar yield and selectivity ratio for **1**:**3** of 64:1.

This desymmetrization procedure increases overall efficiency in preparation of other linkers. The Bednarski procedure is an effective route to azido alcohol **4a** mixed with diazide **2a**. Alcohol **4a** can be efficiently converted to acid azide **5a** by alkylation with potassium bromoacetate in the presence of **2a**. Extraction now serves to separate **5a** from **2a**. Diazide **2a** no longer is wasted, as it can be desymmetrized as described above.



4a **5a**

Significant quantities of these linkers can be prepared with relatively short reaction times and in high purity. No chromatography is needed in these procedures: separations of the amino azides and the azido acid can be achieved through simple extractions. This desymmetrization procedure may prove useful for the preparation of related compounds.

Experimental Section

General Procedures. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. CH_2Cl_2 and Et_3N were distilled from calcium hydride. Et_3N for chromatographic use was freed of ninhydrin-positive contaminants by treatment with 1% acetic anhydride for at least 15 min. THF was distilled from potassium benzophenone ketyl. NMR spectra are 300 MHz in $CDCl_3$ vs TMS.

1,11-Diazido-3,6,9-trioxaundecane, 2a. To tetraethylene glycol (50.78 g, 0.2614 mol), dried by addition and rotary evaporation of 25 mL of toluene, in 200 mL of THF under N_2 was added CH_3SO_2Cl (45 mL, 0.5814 mol) by syringe. The solution was stirred on an ice bath as Et_3N (81 mL, 0.5811 mol) in 50 mL of THF was added dropwise over 27 min, forming a yellow-white precipitate. After 1 h the ice bath was removed, and the mixture was left to stir for 3.5 h with occasional swirling. Addition of H_2O (122 mL) dissolved the solid, forming two liquid phases, which were chilled on a cold water bath, $NaHCO_3$ (12 g, to pH 8) was added followed by NaN_3 (34.88 g, 0.5365 mol), and stirring was started. **WARNING: If azide is added to acid, toxic and explosive HN_3 will form.** Distillation of THF to a solution temperature of 80 °C was followed by reflux for 24 h. The aqueous layer was extracted five times with 100-mL aliquots of Et_2O, and each Et_2O layer was backwashed with the same 50-mL aliquot of saturated NaCl. The Et_2O layers were combined, dried over sodium sulfate, filtered, and concentrated by rotary evaporation, and traces of solvent were removed by evacuation to yield 43.7 g of **2a** as an oil (67%). TLC (silica gel, 1:1 EtOAc:hexanes, $KMnO_4$, $R_f = 0.61$) showed a single component. At smaller scale (≤20 g of tetraethylene glycol) azide substitution carried out at 75−80 °C in H_2O solvent after removal of all THF by rotary evaporation gave diazide **2a** in 85% yield, identical with the material isolated as a byproduct of the first step of Bednarski's procedure.[3] ^{1}H NMR: δ 3.68 (m, 12H), 3.40 (t, $J = 5.1$ Hz, 4H). IR (neat): 2915, 2109 cm^{-1}. MS (EI) M + H calcd 245.1, found 245.1. Anal. Calcd for $C_8H_{16}N_6O_3$: C, 39.34; H, 6.60; N, 34.41. Found: C, 39.33; H, 6.61; N, 34.10.

1-Amino-11-azido-3,6,9-trioxaundecane, 1a. Diazide **2a** (42.7 g, 0.176 mol) was stirred with 400 mL of 0.65 M aqueous H_3PO_4 while Ph_3P (39.9 g, 0.152 mol) in 300 mL of Et_2O was added dropwise over 45 min followed by 35 mL of ether to rinse the addition funnel. After stirring under N_2 for 24 h, the separated aqueous layer was washed with 3 × 100 mL of Et_2O, and 32 g of KOH was added. After traces of ether had evaporated, the mixture was cooled to 4 °C for 16 h, and Ph_3PO was removed by filtration. Addition of 92 g of KOH to the aqueous solution (to 4 M), extraction 16 times with 75-mL aliquots of CH_2Cl_2, drying over Na_2SO_4, filtration, and rotary evaporation gave 30.2 g (82%) of **1a** as an amber oil, spectroscopically identical with an authentic sample.[3] TLC/ninhydrin analysis gave the ratio of **1a**:**3a** as 66:1. ^{1}H NMR: δ 3.68 (m, 10H), 3.52 (t, $J = 5.1$ Hz, 2H), 3.40 (t, $J = 5$ Hz, 2H), 2.87 (t, $J = 5.1$ Hz, 2H), 1.73 (br s, 2H).. FTIR (neat): 3415, 2912, 2106 cm^{-1}. LRMS (electrospray) M + H calcd for $C_8H_{19}N_4O_3$ 219.1, found 219.0. Anal. Calcd for $C_8H_{18}N_4O_3$: C, 44.03; H, 8.31; N, 25.67. Found: C, 43.91; H, 8.07; N, 25.84.

Dilute conditions: Reaction for 14 h of **2a** (52.8 mg, 0.216 mmol) with Ph_3P (51.0 mg, 0.195 mmol) in 2.0 mL of Et_2O with 1.5 mL of 0.1 M aqueous H_3PO_4 gave **1a** without detectable **3a** by TLC. The acidic aqueous layer was washed three times with 3 mL of Et_2O and then made basic with 150 mg of NaOH, and benzyl chloroformate (155 μL, 1.10 mmol) was added. After 14 h, no amine remained (ninhydrin), the mixture was extracted with 3 × 3 mL of CH_2Cl_2, combined CH_2Cl_2 layers were dried over Na_2SO_4 and filtered, and the solvent was removed in vacuo to yield 189 mg of a mixture which was analyzed by HPLC (C-18, 1:1 CH_3CN:H_2O with 0.05% CF_3CO_2H), giving a ratio of 190:1 for **1a**:**3a**.

Mixed-Length Diazide 2. A mixture of poly(ethylene glycol) 200 (Aldrich; 2.20 g, 11 mmol), poly(ethylene glycol) 300 (3.31 g, 11 mmol), and poly(ethylene glycol) 400 (4.42 g, 11 mmol) was treated as for **2a** above (small-scale procedure) to yield 9.7 g (84%) of diazide **2**. ^{1}H NMR: δ 3.67 (m, 41.5H), 3.39 (t, $J = 6$ Hz, 4H).

Mixed-Length Amino Azide 1. Reduction of mixed **2** as described for **1a** was carried out, giving mixed-length **1** in 65% yield. TLC (silica gel, 9:1 CH_3OH:30% NH_3/H_2O, ninhydrin) easily distinguishes amino azides ($R_f = 0.68$) from diamines ($R_f = 0.20$). The ratio of amino azide to diamine was determined by quantitative TLC/ninhydrin analysis to be 64:1. ^{1}H NMR: δ 3.65 (m, 40H), 3.53 (m, 2H), 3.40 (t, 2H), 2.8 (br, 2H), 1.95 (br, 2H).

Statistical Reduction of Diazide 1a. To a solution of diazide **2a** (1.01 g, 4.13 mmol) in 8 mL of CH_3OH was added a solution of $SnCl_2$ (1.05 g, 4.10 mmol) in 3 mL of CH_3OH over 4 min. The mixture was stirred for 14 h, solvent was removed by rotary evaporation, 30 mL of 4 M NaOH was added, and the filtered aqueous layer was extracted 15 times with 10-mL aliquots of CH_2Cl_2 until TLC showed no more amine was extracted. CH_2Cl_2 layers were combined and dried over Na_2SO_4, and solvent was removed in vacuo to yield 0.843 g (93%) of a mixture of **1a**, **2a**, and **3a** as a colorless oil. Quantitative TLC/ninhydrin analysis gave a 2.4:1 ratio of **1a**:**3a**.

Azido Acid 5a. A mixture of azido alcohol **4a** and diazide **2a** prepared as described[3] (11.0 g, 27.8% **4a**, 13.9 mmol) was treated with $BrCH_2CO_2K$ (5.63 g, 31.7 mmol) and KOH (5.94 g, 106 mmol) in 6 mL of DMF at 45 °C for 24 h, after which 6 mL of H_2O was added, and heating was continued for 12 h more to consume excess bromoacetate. After solvent removal in vacuo, the residue in 100 mL of H_2O was washed with 3 × 50 mL of CH_2Cl_2, acidified to pH 2 with solid $NaHSO_4$, extracted with 3 × 60 mL of CH_2Cl_2, dried over Na_2SO_4, and filtered, and solvent was removed in vacuo to yield 3.51 g (91%) of **5a** as a yellow oil. ^{1}H NMR: δ 4.16 (s, 2H), 3.7 (m, 14H), 3.41 (t, $J = 6$ Hz, 2H). FTIR (neat): 3399, 2909, 2107, 1748 cm^{-1}. Anal. Calcd for

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$C_{10}H_{19}N_3O_6$: C, 43.32; H, 6.91; N, 15.15. Found: C, 43.41; H, 7.06; N, 14.82.

Quantitative Ninhydrin Procedure for Amino Azide 1:Diamine 3 Ratio. A solution of ca. 20 mM amine in methanol was applied in 0-, 5-, 10-, 15-, and 20-μL amounts to a silica gel TLC plate. After elution (8:1:1 CH_3OH:H_2O:Et_3N) the silica gel corresponding to each spot was collected, and the amine concentration was determined by a modification of the procedure of Sarin et al.[9] To each sample were added 100 μL of solution A (prepared by dilution of 2 mL of aqueous 10^{-2} M KCN to 100 mL with pyridine followed by addition of 40 g of phenol in 10 mL of ethanol) and 25 μL of solution B (5% ninhydrin in EtOH), and each sample was heated at 100 °C for 10 min, cooled on ice, and diluted to 2 mL with 60% ethanol in H_2O. After centrifugation to remove silica particles, absorbance at 560−570 vs 460−470 nm gave a linear response to [amine]. Amine concentration was determined from the slope by comparison to standard samples.

Supporting Information Available: ^{1}H NMR spectra for compounds **1a** (crude and purified), **2a**, and **5a** and description of purification of analytical samples (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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