Synthesis of Protected Amino Alcohols: A Comparative Study

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The preparation of amino alcohols, from naturally occurring amino acids, has been reported previously using lithium aluminum hydride,[2] sodium borohydride,[3] and borane–dimethyl sulfide.[4] Retention of optical activity was observed in all cases.[5] The preparation of protected amino alcohols, however, and the effect of the reducing procedures on the commonly used protecting groups in peptide synthesis have not, as yet, been reported. Protected amino alcohols can be further utilized as intermediates for the preparation of biologically active peptide and amino aldehydes. Interest in peptide aldehydes has increased recently due to their strong inhibition of proteolytic enzymes.

Lithium aluminum hydride, borane–THF, and DIBAL[6] have been employed for reduction of the N-terminal and side-chain protected amino acids. These methods were compared with respect to yields, optical purity, and stability of protection. The products were characterized by NMR, IR, optical rotation, and TLC in different solvent systems.

Experimental Procedures

The protected amino acids were purchased from Chemical Dynamics Corp. and were characterized prior to their use by melting point and TLC. The reducing agents $LiAlH_4$, borane–THF (1 M in THF), and DIBAL (1 M in toluene) were obtained from Aldrich Chemical Co. Infrared spectra were recorded on a Beckman Acculab instrument and nuclear magnetic resonance spectra (^1H NMR) on a Varian EM-390 in $CDCl_3$. Optical rotations were obtained on a Bausch and Lomb polarimeter in 1-dm cells at 23 °C by using the Na D line. Precoated TLC sheets (silica gel 60F) were purchased from E. Merck. The THF was freshly distilled from $LiAlH_4$ prior to its use.

Reductions Using $LiAlH_4$.[2] The amino acid (13 mmol), dissolved in the minimum required quantity of THF, was added dropwise to the reagent (2.0 g, 52 mmol) suspended in freshly distilled THF (75 mL). The mixture was refluxed for 3 h at 65 °C, and the reaction was then allowed to proceed overnight at room temperature. At this time, the flask was placed in an ice bath, and the reaction was quenched by addition of 0.1 M HCl. The precipitated lithium salts were removed by filtration, and the crude product was recovered from the THF/H_2O mixture by evaporation. The pure alcohol was obtained by passing the crude product through a short silica gel column and eluting with chloroform–methanol (1:1). This solvent system elutes the alcohols first.

Reductions Using DIBAL.[6] The protected ester of the amino acid (5 mmol) was added to toluene (50 mL) and cooled to −30 °C. The DIBAL solution (10 mmol) was added dropwise over a period of 30 min to the ester. After completing the addition, the flask was placed in an ice bath, and stirring was continued for an additional 20 min. The reaction was then quenched by dropwise addition of 2 N HCl and filtered. The aqueous layer was kept and extracted three times (50 mL each) with EtOAc. The product partitioned into the organic phase. The EtOAc was extracted further with 1 N HCl, H_2O, and 1 M NH_4HCO_3, dried over $MgSO_4$, and evaporated to yield the product. For the more polar amino acids THF was used as the reaction medium in place of toluene.[6] Following extraction of the THF/H_2O mixture with EtOAc, the product was recovered from the EtOAc layer as described.

Reductions Using BH_3·THF.[7] A solution of the amino acid (10 mL) in THF was added dropwise to a 0 °C solution of BH_3·THF (20 mL, 20 mmol). The addition occurred over 30 min, and the reaction was allowed to proceed 1–2 h more at 0 °C. After completion of this additional period, the reaction was quenched by using 40 mL of a 10% solution of HOAc in MEOH. After solvent evaporation, the crude product was dissolved in EtOAc and extracted in 1 M HCl, H_2O, and 1 M NH_4HCO_3. The EtOAc layer was dried over $MgSO_4$, and the pure product was recovered in high yields by evaporation of the solvent.

Table I. Properties of Amino Alcohols Prepared by BH_3·THF Reduction

alcohol [a]	% yield	^1H NMR [b]	[α] [c] (concn)
BocGly-ol	44	3.7 (t, 2 H), 1.4 (s, 9 H)	0.00
BocAla-ol	95	3.7 (m, 3 H), 1.4 (s, 9 H)	−1.0 (1.3)
BocVal-ol	95	3.6 (d, 2 H), 1.4 (s, 9 H)	−0.90 (0.5)
BocPhe-ol	60	3.6 (m, 3 H), 1.4 (s, 9 H)	−0.80 (1.1)
BocSer(OBzl)-ol	75	7.4 (s, 5 H), 3.8 (d, 2 H), 1.4 (s, 9 H)	+0.25 (1.0)
BocAsp(OBzl)-ol	75	7.3 (s, 5 H), 3.6 (d, 2 H), 1.4 (s, 9 H)	+0.20 (1.0)

[a] IR showed characteristic bands at 2.9 and 5.9 cm^{-1} for the alcohol and carbonyl stretchs. [b] Only the Boc, side-chain protection, and methylene, of the alcohol are shown. [c] Observed optical rotation; concentration units of g/100 mL of $CHCl_3$.

Results and Discussion

The $LiAlH_4$ and DIBAL reagents, routinely used for the reduction of acids and esters to alcohols, were proven unsuitable for use with protected amino acids and their esters. These reagents were detrimental to the N-terminal protection (*tert*-butyloxycarbonyl) of the amino acids. Apparently the hydride in the lithium aluminum hydride, as well as in the DIBAL reductions, was consumed primarily by the Boc group prior to its utilization for the reduction of the acid/ester function. This was apparent by the extensive (over 80%) deprotection caused, as evidenced by ^1H NMR, and the progressive appearance of a ninhydrin-positive component.

It was originally assumed that the refluxing process (65 °C) in the $LiAlH_4$ reductions was responsible for the removal of the Boc group. Therefore, several reactions were performed in which the reflux time was reduced or eliminated. These studies disproved the initial assumption since N-terminal deprotection was also apparent at room temperature, as well as at 0 °C. The only protected amino alcohols obtained in reasonable yields by the $LiAlH_4$ method were Boc-L-valinol and Boc-L-leucinol.

In the initial attempts, the DIBAL reagent failed to yield the desired product. Several additional reactions were performed by using different amino ester to DIBAL ratios. In addition to the above-mentioned deprotection, severe loss of crude product was observed due to adsorption on the precipitating aluminum salts. Neither of the DIBAL

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or $LiAlH_4$ reductions led to product racemization, in agreement with previous reports.[5]

Best results were consistently obtained by using borane–THF for 1–2 h, depending on the amino acid being reduced. Under the described conditions, the carboxylic acid functions were reduced selectively in the presence of the N-terminal urethane bond. This was evident by the carbonyl IR absorption at 1690 cm^{-1} and the expected 1.4-ppm resonance of the Boc group in the NMR. The properties of some representative amino alcohols prepared by this procedure appear in Table I. All recovered alcohols were shown by TLC to contain a single component, with EtOAc and chloroform–methanol (9:1, 1:1) for plate development. Optical purity was conserved in agreement with studies by Meyers,[5] who demonstrated retention of optical activity when borane–dimethyl sulfide was used to reduce free amino acids to alcohols.

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Synthesis of Nonachloro-3-phenoxyphenol. Carbon-13 Nuclear Magnetic Resonance Spectra of Polychlorinated Intermediates

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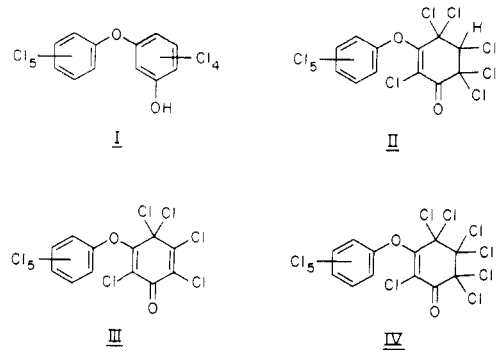
Analysis of technical pentachlorophenol (PCP) shows the presence of numerous chlorinated byproducts which arise in the manufacturing process.[1] The potential health hazards from exposure to these chemicals is of some concern. It has been shown, for example, that nonachloro-3-phenoxyphenol (I), a contaminant of PCP,[2] has a hemolytic potency at least a hundred times greater than that of PCP,[3] and the need for evaluating other toxicological properties of this compound is apparent. A convenient synthetic procedure is, therefore, needed. We report our methods for synthesizing and purifying I.

Results and Discussion

Synthesis of I involved perchlorination of *m*-phenoxyphenol with a combination of sulfur monochloride–aluminum chloride–sulfuryl chloride (BMC reagent).[4] The product mixture from the perchlorination reaction was reduced with sodium iodide to obtain I. The overall yield from starting material, *m*-phenoxyphenol, to I was 37%.

Purification of I was achieved by anion-exchange chromatography followed by extraction of a basic solution of I with 2,2,4-trimethylpentane. Further purification, when necessary, was achieved by chromatography on a reverse-phase RP-18 (25–40 μm) EM-Lichroprep column and



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then on a silica gel Si-60 column as previously described.[5] The purity of the product was determined by HPLC analysis using a 4.0 × 300 mm Waters Associates μ-Bondapak C-18 column and a 4.6 × 250 mm Lichrosorb Si-60 (5 μ) column.

The ^{13}C NMR spectrum of I was obtained in the presence of a spin-relaxation agent, chromium acetylacetonate.[6] The assignment of resonances to the appropriate carbon atoms (Table I) was facilitated by preparing the methyl ether derivative and comparing changes in chemical shifts, $\Delta\delta$, for each carbon atom. A similar comparison was made in Table I for PCP and pentachloroanisole (PCA). The spectrum of I showed two peaks (δ 131.4 and 124.5) that were approximately twice as intense as the rest. These are comparable to those of decachlorodiphenyl ether (δ 131.3 and 123.9). The comparison with diphenyl ether and the observation that carbons β to an oxygen are shifted upfield of benzene (δ 128.5)[7] resulted in the assignments C2′–C6′. Because of the large internuclear distance between these carbon nuclei and the hydroxy group in the adjacent ring, small $\Delta\delta$ values were expected on methylation of the hydroxy group. This was observed (Table I).

The three resonances farthest downfield are clearly associated with oxygen linkages. Comparison with $\Delta\delta$ values for PCP/PCA (Table II) led to the assignment of C1 in I. C1′ and C3 were assigned on the basis of a larger $\Delta\delta$ for the carbon (C3) closer to the site of substitution.

A relatively small $\Delta\delta$ (1.2 ppm) on conversion of the hydroxy to a methoxy group led to the assignment of C5. This value is similar to that observed for the meta carbon atom in PCP/PCA and is consistent with previous reports that meta carbons are not influenced greatly by replacing a hydroxy with a methoxy group.[7] In contrast, ortho and para carbons are shifted significantly by this substitution. The para carbons in PCP and PCA have a $\Delta\delta$ of 5.8 ppm. A similar value ($\Delta\delta$ = 6.2 ppm) for I compared to its methyl ether was used to assign C4. C2 is β to two oxygens, and it was assigned the farthest upfield chemical shift (113.3 ppm). C6, therefore, must appear at 118.3 ppm.

Gas chromatographic-mass spectrometric analysis of the oily residue from perchlorination of *m*-phenoxyphenol indicated the presence of numerous compounds including PCP, hexachloro-2,5-cyclohexadienone, hexachlorobenzene, and chlorinated derivatives with apparent molecular ions (first peak of the isotope clusters) of *m/z* 562 (II), 526 (III), and 596 (IV). Mass spectral analysis of the mixture via the probe also showed chlorine clusters with masses above *m/z* 680. When II was isolated and reduced by sodium iodide in methanol–hexane solution, I was obtained in 85% yield.

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