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EXPEDITED ISOLATION IN THE SYNTHESIS OF PEPTIDES BY ION EXCHANGE

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We wish to report the initial development of an ion exchange procedure which provides a potential solution to the failure sequence problem[1] in the expedited synthesis of peptides. Notwithstanding the continuing refinement of the solid phase method and variants thereof,[2] all such approaches that depend on an attached "handle" for rapid isolation are incapable of discriminating between desired product and untransformed starting material. Thus, the applicability of handle strategies in stepwise synthesis ultimately must be limited by the accumulation of impurities in successive reactions. To overcome this limitation our method is, instead, based on isolation operations which distinguish the products of repetitive coupling and deprotection reactions from the precursors by virtue of their neutrality and basicity,[3] respectively.

For the coupling step work up the doubly protected product is passed selectively, as the only neutral non-volatile component of the reaction mixture, through a mixed bed column of ion exchange resins in the Et_3NH^+ and OAc^- forms,[7] and evaporation of solvent and discharged Et_3NH^+,OAc^- leaves the desired product. Although our initial study has been restricted primarily to mixed anhydrides (decomposing excess acylating agent to carboxylic acids), this general procedure should prove useful with a variety of available coupling reagents for which excess reagents, co-products, and by-products are acidic, basic, ionic, or volatile. In a series of test couplings, the hydrochloride of the amine component (or free $PhCH_2NH_2$) and Et_3N was added to a $\sim$0.1 M DMF solution of a 2-fold excess of the mixed anhydride (formed at -40°) of Me_3CCO_2H and the N-protected amino acid. After the reaction mixture had

warmed, water was added (cloud point or 25-75%), and the solution was heated (steam bath) and passed down a column of 10-15 equiv. each of Bio-Rad Ag-1-X2 (as OAc^-) and AG-50W-X2 (as Et_3NH^+) resins with more DMF·water (same composition).[12] In all cases evaporation of the combined eluent and column rinse left > 90% of the crystalline product. ZGly-$NHCH_2Ph$ (yield 96%, mp 117-118°, rcrd mp 120-121°), ZGly-GlyOEt (90%, 79-80°, 81-83°), ZGly-Gly_2OEt (100%, 161-165°, 165-167°), ZGly-AlaOEt (91%, 63-65°, 65-66°), ZPhe-$NHCH_2Ph$ (95%, 147-154°, 161-163°), ZPhe-AlaOEt (93%, 116-120°, 123-124°), BOCLeu-LeuOMe (98%, 131-134°, 138-139°), and BOCLeu-GlyOEt (95%, 79-80°, 80-82°).

The deprotection product, after N-BOC removal by stirring in TFA and evaporation with MeCN, is selectively retained on a cation resin,[13] as the only non-volatile cationic component of the reaction mixture. Capture on a Et_3NH^+ resin was examined first, with elution by Et_3NH^+, OAc^- and evaporation to give the product acetate salt (or the chloride by addition of 1 equiv. of Et_3NH^+,Cl^- prior to evaporation or by passage of the acetate through a Cl^- column). While this technique warrants further evaluation, in some cases inefficient evaporative removal of Et_3N led to decomposition of base-sensitive dipeptides. To provide an isolation suitable for even such sensitive cases, retention of the ester from a solution of the TFA salt in an appropriate solvent (1:1 acetone/water in the tests below) with a column of 6-10 equiv. of Bio Rad AG-50W-X2 (as Na^+) and elution with excess aqueous Na^+,OAc^-[12] was substituted for use of Et_3NH^+,OAc^-. In this modification, employed throughout the tests below, the product acetate salt is selectively dissolved from the eluent evaporation residue with DMF and converted to the chloride by passage through a Cl^- exchange column prior to the next coupling.

To illustrate the utility of the techniques in combination, a series of small peptides were synthesized in stepwise fashion with no intermediate work up other than by the above ion exchange methods. In all cases crystalline final products were obtained directly, and overall crude yields correspond to average conversions of about 90% per reaction [15] ZGly$_3$OEt (76%, 142-145°, 157-162°), ZGlyLeu$_2$OMe (59%, 123-126°, 133-134°), ZGly$_4$OEt (53%, 197-202°,

213-214°), Z(GlyLeu)$_2$OMe (55%, 80-83°, 91-92°), Z(GlyLeuGly)$_2$OMe (40%, 151-165°, 188-189°).

Despite the preliminary nature of the results, advantages are demonstrated in general for strategies that do not depend on a handle for expedited isolation. First, the use of different isolation operations in alternation after successive reactions can serve as an effective barrier to the carry-over of other impurities besides untransformed starting materials. For example, the incorrect mode of acylation with the present coupling agent would form neutral, non-volatile pivalamides that would survive coupling isolation. However, traces of these impurities, detectable by nmr, are uniformly removed in the following deprotection step. The more serious anticipated hazard would be impurities that remain indistinguishable from the growing peptide chain in succeeding reactions and, therefore, never are removed. Side reactions that convert the acylating agents to neutral derivatives of the N-protected amino acid constitute a problem of this type. In the present work in DMF, acylation of dimethylamine forms traces of dimethylamides as one such impurity. Nevertheless, the population of error sequences resulting from participation of these accumulating impurities in later reactions is akin to the situation with truncated error sequences and should be much less serious than the failure sequence problem. Moreover, most impurities in this category would be easily removed from the ultimate desired product by simple procedures. As an illustration, the crude protected hexapeptide above was freed of contaminating amides by selective basic hydrolysis of the ester group, retention of the free acid on an OAc^- ion exchange column, elution with excess aqueous HOAc, and evaporation - leaving material in a good state of purity (1° mp depression).

Finally, our initial tests suggest the ion exchange procedure may prove a particularly useful non-handle approach. The basic method lends itself to extension to other coupling agents and less water-rich media. Other good features are that reactions are carried out in solution, assay and/or auxiliary purification of intermediates is convenient, the ion exchange columns are regenerable appliances, and recovery of excess amino acids is possible.

REFERENCES

1. E. Bayer, H. Eckstein, K. Hagels, W. A. Konig, W. Bruning, H. Hagenmaier, and W. Parr, J. Amer. Chem. Soc., 92, 1735 (1970).

2. Recently reviewed by E. Wünsch, Angew. Chem., Internat. Ed., 10, 786 (1971).

3. Acid-base extraction schemes have previously exploited this difference in properties[4-6], but the limited utility of extractive techniques (restricted to water-immiscible solvents) has been noted.[6] Adoption of ion-exchange methods permits extension of acid-base separations to more useful, polar solvents.

4. K. Medzihradsky and J. Radoczy, Proc. European Peptide Symp., 6th, Athens, 1963, 1. 49.

5. J. C. Sheehan, J. Preston, and P. A. Cruickshank, J. Amer. Chem. Soc., 87, 2492 (1965).

6. C. H. Schneider and W. Wirz, Helv. Chim. Acta, 55, 1065 (1972).

7. Less mild ion exchange conditions have been used previously to remove non-neutral contaminants in the special case of peptide amide purification[8-10] and reverse stepwise synthesis with ionic carbodiimides.[11]

8. R. Sarges and B. Witkop, J. Amer. Chem. Soc., 87, 2020 (1965).

9. S. Makisuma, M. Waki, and N. Izumiya, Bull. Chem. Soc. Japan, 44, 158 (1971).

11. D. G. Knorre and T. N. Shubina, Dokl. Akad. Nauk SSSR, 150, 559 (1963), Acta Chim. Acad. Sci. Hung., 44, 77 (1965).

12. All ion exchange columns had height to diameter ratios > 6. For 1 mmole scale reactions flow rates from ∿ 3 ml/min to ambient were employed. Elution progress was checked by monitoring non-volatile residues, the column operations in general resulting in approximately 10-fold dilution.

13. Previous ion-exchange capture of deprotection products has employed strongly acidic resins and basic eluents of less general utility.[4,14]

14. C. J. Gray and A. M. Kahoujah, Tetrahedron Letters, 2657 (1969).

15. Mp and rotations for the once-recrystallized products in all cases were equal or superior to literature values.