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Hydrolysis Of Polypeptide Esters with Tetrabutylammonium Hydroxide

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Abstract: Tetrabutylammonium hydroxide is effective in hydrolysis of polypeptide esters to the corresponding acids with minimum racemization of the stereogenic centers at the α-positions. It is especially effective in hydrolysis of non-polar polypeptide esters that are insoluble in most common solvents.

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In a conventional polypeptide synthesis, the chain is propagated from the amine terminus while the terminal carboxyl group remains protected throughout the synthesis. The choice to propagate the chain from the amine side is due to the relative ease of protection and deprotection of amines compared to acids. Carboxylic acids are commonly protected as esters. Cleavage of esters can be accomplished under different conditions, however, base catalyzed hydrolysis is most common. Since most polypeptide esters contain stereogenic centers at the α-carbons, basic hydrolysis may be accompanied by racemization at this position, a problem that is compounded as the length of the polypeptide chain increases, primarily due to solubility limitations. The literature contains several alternative procedures for cleavage of esters such as alkali metal carbonates in alcoholic solvents.[1] Other procedures[2] include displacement of the ester alkyl group by nucleophiles such as alkyl thiolate,[3] phenyl selenide,[4] chloride,[5] *tert*-butoxide[6] ions, or $(CH_3)_3SiI$[7] in aprotic solvents. When applied to polypeptide esters, many of these methods are not suitable for large scale and suffer from side reactions. Alkali metal hydroxides or carbonates remain the preferred reagents for hydrolysis of polypeptide esters.

In our work on the liquid phase synthesis of polypeptides, we required hydrolysis conditions that could be used on large scale with minimum loss of stereochemical purity. Such conditions were to be applied to hydrolysis of polypeptide esters of 8 or more amino acid residues in non-conventional convergent syntheses in which a polypeptide chain propagates from the carboxy terminus. The use of tetrabutylammonium hydroxide[8] as alternative to alkali metal hydroxides satisfied most of these requirements. Tetraalkylammonium hydroxides are strong bases which to date are used mainly in hydrolysis of amides[9] and occasionally esters[10] and thioesters.[11] They possess good solubility in water and in many organic solvents which may be necessary for reactions with water-insoluble molecules. For all cases reported herein, we used tetrabutylammonium hydroxide (n-Bu_4NOH) which is available commercially as 40% aqueous solution; however, other tetraalkylammonium hydroxides may be used. We present our preliminary results from a study directed at the hydrolysis of polypeptide esters using n-Bu_4NOH in this report in Table 1.

The liquid phase convergent synthesis of $(LysLeu_4)_4Lys$ (known as KL_4), a pulmonary surfactant synthetic protein,[12] involves the synthesis of the acid fragment Boc-Lys(Z)-Leu_4-Lys(Z)-Leu_2-OH (**2**) to be used in a key step.[13] Attempts to hydrolyze the methyl ester **1** with aq LiOH, NaOH or KOH in solvents such as MeOH, acetone, THF, THF/MeOH resulted in slow reactions and the formation of high levels (10-15%) of the diastereomer resulting from epimerization at the ester α-carbon (named D-8 diastereomer). Initially, we

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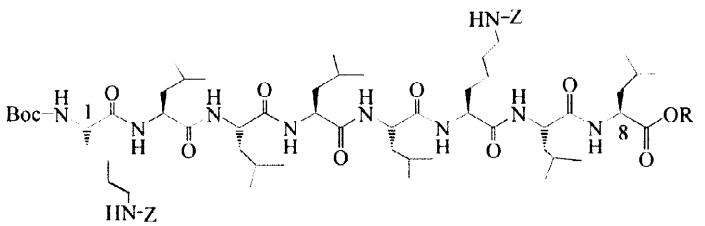
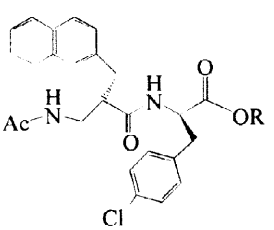
developed improved hydrolysis conditions by using aq NaOH in DMF which gave a good yield of the acid with less (5-6%) of the D-8 diastereomer (Table 1, entry 2). *The NaOH/DMF system is a very effective and convenient procedure that should be considered in hydrolysis of polypeptide esters*, but in this case, the level of the epimerization was too high. The poor solubility of **1**, in most solvents, was probably the main reason for slowness of the reaction and consequently the high levels of epimerization. This problem was overcome by the use of *n*-Bu_4NOH. Hydrolysis of **1** with aq 40% *n*-Bu_4NOH in DMF or THF at -5 to 0 °C gave the acid **2** in high yield and low epimerization. In a typical reaction, 2-3 equivalents of the base is added slowly to the suspension of the powdered solid ester in THF at -5 to 0 °C; there is a noticeable remarkable solublizing effect from *n*-Bu_4NOH which results in fast dissolution and formation of a clear light yellow solution. The progress of the reaction is followed by HPLC analysis and hydrolysis is complete in about 1.5h. The reaction must be kept at low temperature (below 5 °C); higher temperatures may result in decomposition of the product or increase in the undesired diastereomer. Acidification with aq AcOH[14] and evaporation of most of the THF gives the acid **2** in 94% yield containing only about 0.8% of the D-8 diastereomer (Table 1, entry 1).



1. R = Me
2. R = H

We evaluated the general use of *n*-Bu_4NOH in hydrolysis of other polypeptide esters. Small polypeptide esters such as Boc-Leu-Phe-OBzl and Boc-Leu-Leu-Leu-Leu-Lys(Z)-OBzl are hydrolyzed equally well using either *n*-Bu_4NOH or traditional conditions such as NaOH in aq acetone. Both reaction conditions give high yields of the corresponding acids with low epimerization (Table 1, entries 3-6). There are, however, some differences. Reactions with NaOH occur at a reasonable rate at rt but proceed very slowly at 0 °C while those with *n*-Bu_4NOH are fast at or below 0 °C. The best results with *n*-Bu_4NOH were obtained in 2 : 1 acetonitrile/water on 0.2-2.1 mmol scale and the hydrolysis was complete in <10 min at 0 °C

As the length of the polypeptide chain increases, its solubility in most organic solvents decreases and the hydrolysis with *n*-Bu_4NOH becomes clearly the method of choice. This is demonstrated in the hydrolysis of Boc-Leu_6-OMe and Boc-Leu_8-OMe at 0 °C (Table 1, entries 7 & 9). The esters completely dissolved and were hydrolyzed in 2h or less giving high isolated yields and low epimerizations. On the other hand, hydrolysis with aq NaOH in acetone proceeded very slowly and needed 6 days to reach 87% conversion. The only good results with aq NaOH were obtained in DMF at rt (Table 1, entries 8 & 10), however, reactions were slower and gave slightly higher diastereomer content.



3. Ac-D-Nal-D-*p*-Cal-OMe, R = Me
4. Ac-D-Nal-D-*p*-Cal-OH, R = H

This hydrolysis procedure was applied to compounds **3** and **5** in the course of liquid phase synthesis of Azaline B, a decapeptide with several unnatural amino acid residues. The hydrolysis of the dipeptide ester Ac-

D-Nal-D-*p*-Cal-OMe (**3**) with aq NaOH/acetone (Table 1, entry 11) gave a product which was contaminated with about 9% (HPLC area %) of the diastereomeric impurity resulting from epimerization at the α-carbon of the ester. The hydrolysis was greatly improved by the use of *n*-Bu_4NOH, the diastereomeric impurity was decreased to 1.6% and the HPLC purity of the isolated acid (**4**) was >95% (Table 1, entry 12).

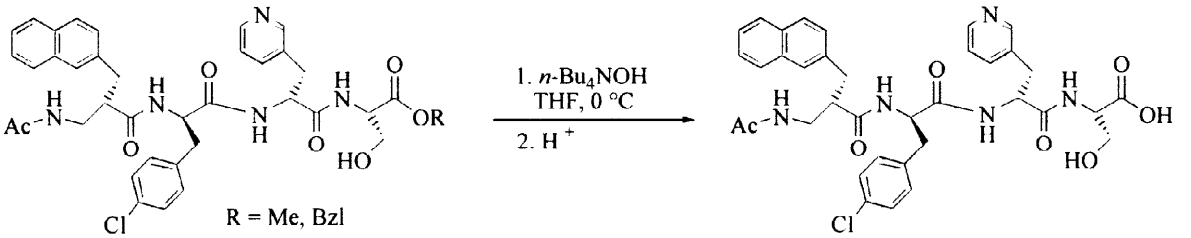
Table 1: Hydrolysis of Polypeptide Esters

entry	Polypeptide Ester	Reaction Conditions	Diastereomeric Ratio[1]	Yield of Acid
1	Boc-Lys(Z)-Leu_4-Lys(Z)-Leu_2-OMe	*n*-Bu_4NOH (2.5 eq), THF/H_2O (18:1), 0 °C, 1.5h	99.2 : 0.8	94%
1	Boc-Lys(Z)-Leu_4-Lys(Z)-Leu_2-OMe	NaOH (2-3 eq), DMF/H_2O (26:1), rt, 3h	94.0 : 6.0	82%
3	Boc-Leu-Phe-OBzl	*n*-Bu_4NOH (1.5 eq), CH_3CN/H_2O (3:1), -15 °C, 10 min	99.7 : 0.3	90%
4	Boc-Leu-Phe-OBzl	NaOH (1.5 eq), rt, acetone/H_2O (1:1), 30 min	99.4 : 0.6	95%
5	Boc-Leu_4Lys(Z)-OBzl	*n*-Bu_4NOH (1.5 eq), CH_3CN/H_2O (24:1), -15°C, 10 min	99.9 : 0.1	98%
6	Boc-Leu_4Lys(Z)-OBzl	NaOH (2.5 eq), rt, acetone/H_2O (4:1), 30 min	99.9 : 0.1	75%
7	Boc-Leu_6-OMe	*n*-Bu_4NOH (2 eq), DMF/H_2O (33:1), 0 °C, 2h	99.6 : 0.4	90%
8	Boc-Leu_6-OMe	NaOH (2 eq), DMF/H_2O (26:1), rt, 7h	99.0 : 1.0	80%
9	Boc-Leu_8-OMe	*n*-Bu_4NOH (2 eq), THF/H_2O (31:1), 0 °C, 2h	98.7 : 1.3	92%
10	Boc-Leu_8-OMe	NaOH (3 eq), DMF/H_2O (33:1), rt, 7h	98.3 : 1.7	82%
11	Boc-D-Nal-D-*p*-Cal-OMe	NaOH (2-3 eq), acetone/H_2O (10:1), rt, 1.5h	91.2 : 8.8	91
12	Boc-D-Nal-D-*p*-Cal-OMe	*n*-Bu_4NOH (2.5 eq), THF/H_2O (14:1), -5 °C, 40 min	98.4 : 1.6	89
13	Ac-D-Nal-D-*p*-Cal-D-3-Pal-L-Ser(OH)-OBzl[2]	(a) NaOH (6 eq), rt, acetone/H_2O (4:1), 24h. (b) Hydrogenolysis	multiple products. slow, side products.	--- ---
14	Ac-D-Nal-D-*p*-Cal-D-3-Pal-L-Ser(OH)-OR	*n*-Bu_4NOH (1.5-2 eq), THF/H_2O (33:1), -5°C, 1.5h (R=Bzl), 0.5h (R=Me)	98.3 : 1.7	82%

(1) Ratio of product to diastereomers resulting from racemization at α-carbon of the ester in crude reaction mixture determined by HPLC area% analysis.
(2) Nal = 2-Naphthylalanine, *p*-Cal = *p*-Chlorophenylalanine, 3-Pal = 3-Pyridylalanine.

Hydrolysis of Boc-D-Nal-D-*p*-Cal-D-3-Pal-L-Ser(OH)-OR (**5**, R = Bn) with NaOH in aq acetone at rt was a very slow reaction (Table 1, entry 13). After 24h, some dissolution occurred and the HPLC analysis showed the formation of multiple products. Using up to 6 equivalents of NaOH did not improve the reaction. Attempts to remove the benzyl group via hydrogenolysis was very slow reaction and resulted in considerable epimerization, partial reduction of the pyridine ring and recovered unreacted ester. The hydrolysis of the

benzyl ester with n-Bu_4NOH (1.5-2 eq.) at -5 °C in THF gave the acid cleanly and in high diastereomeric purity. The HPLC analysis shows complete conversion in about 1.5h. The product was isolated after acidification with aq AcOH and purification with hot MeOH, in 82% yield containing only 1.7% of the undesired diastereomer (DDDD-diastereomer). The hydrolysis of the corresponding methyl ester (**5**, R = Me) was even faster (0.5h); it gave, after work-up, 82% yield of isolated purified solid identical to that obtained from hydrolysis of the benzyl ester (Table 1, entry 14).



(**5**) Ac-D-Nal-D-*p*-Cal-D-3-Pal-L-Ser(OH)-OR (**6**) Ac-D-Nal-D-*p*-Cal-D-3-Pal-L-Ser(OH)-OH

The results of this investigation, thus far, indicate that tetrabutylammonium hydroxide is a useful reagent for hydrolysis of polypeptide esters. In some cases, such as the hydrolysis of **5**, it is the only reagent that could cleanly effect this hydrolysis. The examples presented herein demonstrate the importance of the solublizing effect of n-Bu_4NOH we first noticed in the KL_4 synthesis. Its use in hydrolysis reactions gives a clear advantage over the traditional procedures with polypeptide esters that are insoluble in water and most common organic solvents. This procedure provides a mild and effective way to carry out ester hydrolysis with minimum racemization of the stereogenic centers at the α-position of the esters. The use of aqueous NaOH in DMF at rt provides another effective and convenient hydrolysis procedure that may be applied in many cases.

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14. 1N HCl is used in most cases for acidification, however, with compounds containing Boc-protective or other acid-sensitive groups, this should be done carefully at low temperature. Alternatively, aq. AcOH is used for acidification to avoid such complication.