Sodium–Liquid Ammonia Reduction in Peptide Chemistry†

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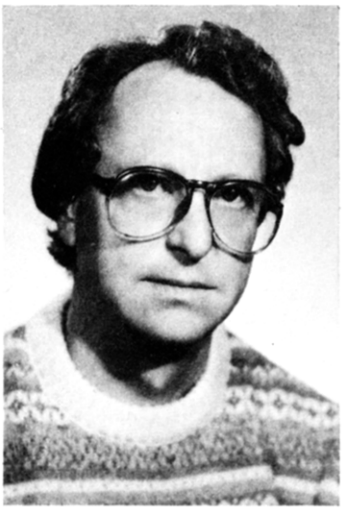

István Schön was born in Budapest, Hungary, in 1942 and graduated in organic chemistry at the Eötvös Loránd University of Sciences in 1967 and then joined the peptide chemical division of the Chemical Works of Gedeon Richter, where he has worked in Academician Kisfaludy's group to the present. From 1973 he attended a 2-year postgraduate course on pharmaceutical research and obtained a university doctor's degree in organic chemistry at the Technical University of Budapest. In 1978 he obtained a C.Sci. degree in chemistry from the Hungarian Academy of Sciences and spent 1 year as a Research Fellow in Dr. Gross' laboratory at NIH, Bethesda. The major topics of his publications are syntheses and structure–activity relationship of biologically active peptides. His main interest is the methodological research and development of the peptide chemistry with special emphasis on side reactions and their potential utilization.

I. Introduction

The introduction of sodium–liquid ammonia reduction for the removal of benzyl-type and tosyl protecting groups was an essential milestone in the history of peptide chemistry about 50 years ago[1-3] and significantly contributed to its speedy development resulting in the synthesis of carnosine[2] in 1935, glutathione[4] in 1936, oxytocin[5-9] in 1954, lysine-[10-13] and arginine-vasopressins[14-17] in 1957. Over three decades this method was indispensable in the synthesis of peptides containing cysteine residues. Vincent du Vigneaud, the Nobel Prize Laureate of 1955 in chemistry, rendered an imperishable service in this work.

Though the recognition of undesired side reactions drew attention to the limits of its applications, the sodium–liquid ammonia reduction is still a well-established protocol for the synthesis of analogues of oxytocin[18] and the vasopressins,[19] and its advantages over acidolysis with hydrogen fluoride were recently emphasized in the last step of the synthesis of human β-endorphin consisting of 31 amino acid residues.[20]

Although a number of excellent textbooks[21-28] deal from different points of view with some aspects of the application of sodium–liquid ammonia reduction in the field of general organic and peptide chemistry, no comprehensive review on this topic has been hitherto published. The scope of this review comprises the results of sodium–liquid ammonia reduction in the removal of protecting groups in peptide chemistry with special emphasis on side reactions. Some data obtained in other fields, e.g., carbohydrate, nucleotide, heterocyclic chemistry, etc., are included. Recent findings[29-31] may give a new impetus to the application of sodium–liquid ammonia reduction in peptide chemistry as an alternative method to the generally used acidolysis in the final deprotection stage as well as in other fields of organic chemistry.

The abbreviations used for amino acids and the designation of the substitutions conform to the IUPAC rules. Except for glycine, all amino acid residues are of L configuration if not otherwise stated. In addition Bzl stands for benzyl, Z for benzyloxycarbonyl, Boc for *tert*-butoxycarbonyl, Tos for (4-methylphenyl)sulfonyl (tosyl), Ac for acetyl, ^{t}Bu for *tert*-butyl, Me for methyl, Cha for cyclohexylamine, Hse for L-homoserine (L-2-amino-4-hydroxybutyric acid), and Trt for triphenylmethyl (trityl).

The present review covers the literature through October 1983.

II. Initial Applications in Peptide Chemistry

The sodium–liquid ammonia reduction was introduced to organic chemistry in the 1920s, when its general applicability as a reducing agent was demonstrated.[32,33] The reaction between organic sulfur compounds

† Dedicated to the 60th birthday of Lajos Kisfaludy, academician.

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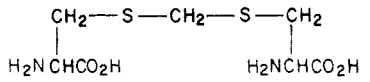
and the reagent was extensively investigated.[34] Reduction of the S–S linkage of cystine to the mercapto group was simultaneously reported from two independent laboratories[1,35] in 1930. This is the first example of the application of this reduction method in peptide chemistry.

The S-benzylation of cystine was accomplished by adding benzyl chloride to sodium–liquid ammonia reduced cystine.[1,36] Similar procedures were developed for S-methylation[37,38] and ethylation[39] of thiols. Djencolic acid (1) was prepared in the same way by using



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dichloromethane.[40] The removal of the *N*-benzyloxycarbonyl group[41] was reported in the synthesis of L-carnosine (H-β-Ala-His-OH)[2] and $(Cys)_2$-Gly_2.[42] The recognition that the *S*-benzyl group was cleaved from *S*-benzylcysteine by sodium–liquid ammonia reduction[2] cleared the way for the synthesis of peptides containing cysteine residues. In the last step of the synthesis of glutathione [H-Glu(Cys-Gly-OH)-OH], the *N*-benzyloxycarbonyl and *S*-benzyl groups were simultaneously removed.[4] Preparation of cystine,[43] homocystine,[44] and homocysteine derivatives[45–48] was perfected by sodium–liquid ammonia reactions. The N-benzylation of histidine for the protection of the imidazole ring and the removal of *N*-tosyl group by sodium in liquid ammonia were reported in 1937.[3]

The effect of sodium on amino acids,[49,50] piperazine-2,5-dione,[50] proteins,[50–53] and parathormone[54] was thoroughly studied in the 1930s. The generation of hydrogen gas was observed in these reactions. The statement that "the peptide linkage in dipeptides is not acidic"[51] relative to the reagent can be considered one of the most essential conclusions of these experiments from the point of view of the future applications as was shown in the reactions of H-Gly-Gly-OH and H-Gly-Ala-OH with sodium.

III. Side Reactions during the Deprotection of Peptides

du Vigneaud had hardly given an account of the reduction of cystine to cysteine at the meeting of the American Society of Biological Chemists in 1930[1] before Gebauer-Fuelnegg reported that some sodium sulfide and alanine had been also formed in the course of the reaction.[35] When solutions of individual amino acids in liquid ammonia were treated with sodium to prepare sodium salts, several products were produced from asparagine.[49] These experimental observations indicated that the application of sodium–liquid ammonia reduction would not be exempt from problems.

A. Racemization

Several data were reported on the racemization of peptides during the sodium–liquid ammonia reduction. It was shown that in the acid hydrolysate of polyglutamic acid, resulting from the sodium–liquid ammonia reduction of the polybenzyl ester, the extent of racemization was approximately 50%, whereas after the reduction with phosphonium iodide no racemization was detected.[55] In an attempt to prepare the free tripeptide H-Met-Met-Met-OH by reduction of the *N*-benzyloxycarbonyl derivative, a totally racemized product was obtained.[56] In this experiment the *S*-methyl groups were also partially cleaved during the reduction and were subsequently remethylated by addition of methyl iodide. There is no reasonable explanation for this complete racemization as the same treatment of the appropriate dipeptide derivative resulted in an optically pure product. Racemization was reported to occur during the reduction of Z-Cys(Bzl)-Val-OH and in lesser extent of H-Cys(Bzl)-Val-OH,[57] but these data were not supported by the necessary experimental evidence.

The reduction of DL-methionine to DL-homocysteine was reported in 1941.[58] Later this method was used to synthesize sulfides derived from DL-homocysteine.[59–61] The reduction of the L enantiomer led to a significant racemization under the same conditions.[62] If the reduction were accomplished in the presence of a proton donor (e.g., methanol, ethanol, or water) or after the reduction step ammonium chloride were added to the reduction mixture in order to liberate the sodium salt, the racemization was nearly and completely suppressed, respectively.

In a thorough and brilliant series of subsequent experiments, Hope unequivocally showed that sodium amide is responsible for the racemization.[63] The reduction mixture of methionine was kept at −33 °C for 24 h. After the workup the resulting L-homocysteine was optically pure. However, when ammonia was evaporated and the residual mixture of sodium amide and the disodium salt of L-homocysteine was kept at room temperature, the rotations of homocysteine samples isolated at various intervals showed a progressive decline. When the reduction was carried out with potassium and the mixture was kept at −33 °C for 24 h, complete racemization was detected. This difference stems from the different solubility of sodium amide (<0.2 g/100 mL)[64,65] and potassium amide (>45 g/100 mL)[66,67] in liquid ammonia at the boiling point. Thus, *the presence of the strong base amide ion mediates racemization by α-proton abstraction.*[63] Therefore, in some cases the reduction of protected peptides was accomplished in the presence of weak proton donors (e.g., acetamide[68] or urea[69]) to destroy the amide ion formed.

B. Damage of Individual Amino Acid Residues

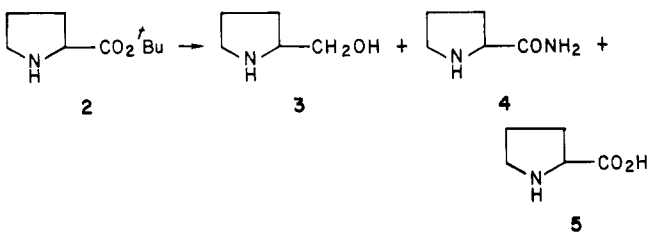
Short references can be found in the literature on the decomposition of tosylserine[70] and tosylthreonine[71] during reduction. The formation of glycine was detected in the reduction mixture of the former.[72] The damage of asparagine,[49] the sensitivity of threonine,[73] and the complete decomposition of a C-terminal tryptophan hydrazide[74] were reported as well. The latter observation may not be connected with the indolylmethyl side chain because the sodium–liquid ammonia reduction was successfully applied for the debenzylation of *N*-benzylindoles[75] and for the synthesis of mercaptoindoles from the *S*-benzyl derivatives.[76] Though the partial saturation of pyrrole[77] and the benzene ring of indole derivatives[78] were also reported by the action of sodium in ammonia, this side reaction may be negligible

in peptide chemistry.[79] The partial reduction of the phenyl group of phenylalanine was assumed[80] as sodium in liquid ammonia is capable of reducing aromatic systems.[81,82]

The formation of alanine was observed not only in the reduction of cystine[35] but also in that of cysteine derivatives.[74,83–86] In some cases the cleavage of *N*-tosyl group was not complete[87,88] or very slow.[89] The formation of ornithine was detected in the reduction of peptides containing an arginine residue.[90] Chinese researchers synthesized insulin using nitroarginine.[91] It was stated that a significant decomposition of arginine was due to the long treatment with a high excess of sodium. Though the demethylation of methionine was early shown,[58,62,63] the reduction process has been applied to the deprotection of peptides containing methionine residues.[56,92–96] It is not surprising that this reaction might partially account for the yield of 20% when a large excess of sodium was used.[94] Acyl migration was observed in peptides containing a 2,4-diaminobutyric acid residue.[96,97]

C. Sensitivity of Proline. Cleavage of the Peptide Bond

It is worth dealing with the sensitivity of proline derivatives in a separate section because of the ample availability of data. Ramachandran showed that the reduction of proline *tert*-butyl ester (**2**) resulted in prolinol (**3**), prolinamide (**4**), and proline (**5**).[98] Under



2 3 4 5

the same conditions the N-acetylated **2** transformed to 5-(acetylamino)valeric acid (**6**, R = OH) and its *tert*-butyl ester (**6**, R = O^{t}Bu) via the opening of the pyr-

$$AcNH(CH_2)_4COR$$
6, R = OH, O^{t}Bu

rolidine ring. While the *tert*-butyl ester group has a tendency to be reduced, hydrolyzed, and amidated during the sodium–liquid ammonia reduction, it is interesting to note that the *N-tert*-butoxycarbonyl group is considered to be stable under the same conditions.[69,99–101]

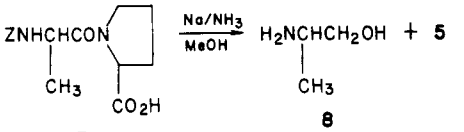
Partial cleavage of the Thr–Pro bond was observed during the preparation of semisynthetic and synthetic insulins.[85,91,102–108] A glass apparatus was devised for the titration of a solution of protected peptides in ammonia with a solution of sodium in ammonia in order to avoid the high local excess of sodium.[104] The partial cleavage of the Lys–Pro bond was observed in the synthesis of α-melanotropin, α-adrenocorticotropin, and their analogues.[95,109–112] It was stated that the cleavage of the peptide bond was due to the very drastic conditions of the reduction, and the yield of the reduction step decreased with the number of the protecting groups to be removed.[110,111] Guttmann showed that the Lys–Pro bond cleavage resulted from the presence of water and other proton donors.[90] The reduction of acyl-Lys(Tos)-Pro-OH and acyl-Lys(Tos)-Pro-NH_2 gave homogeneous products.[74]

Recently, Japanese researchers reported 20–30% cleavages of Asn–Pro and Leu–Pro bonds during sodium–liquid ammonia reduction of the protected C-terminal 24-peptide of thymosin-β_4.[113] The lability of the peptide bonds of a model peptide Z-Asn-Pro-Leu-Pro-Ser-OH (thymosin-β_4 26–30 segment) was in accord with that of the 24-peptide. On the other hand, the reductions of Z-Asn-Pro-OH and Z-Leu-Pro-OH resulted in the expected dipeptides without significant side reactions under the same conditions.

From these contradictory observations it seems that minute modifications in the same experimental technique and even local customs of the individual laboratories might have a significant influence on the results and their interpretations.

In model experiments Marglin studied the factors influencing the partial cleavage of the Thr–Pro bond in various protected and unprotected derivatives of the C-terminal sequence of the insulin B chain, Thr-Pro-Lys-Ala.[114] It was stated that the extent of peptide bond cleavage did not depend on the different numbers of protons released into liquid ammonia by the functional groups surrounding the sensitive peptide linkage. The partial cleavage of the Thr–Pro bond could be minimized by limiting the excess of sodium and the duration of the treatment. The sensitivity of Ser–Pro,[114] Leu–Pro,[80] and to a lesser extent of Val–Pro[114] and His–Pro[90] bonds have also been reported.

Ressler and Kashlikar showed that the Birch reduction of Z-Ala-Pro-OH (**7**) in the presence of methanol led to the complete reduction of the acyl moiety to alaninol (**8**) and cleavage of the peptide bond.[80] Similar



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treatment of Z-Leu-Pro-Gly-OH resulted in the formation of L-leucinol. Aliphatic tertiary amides may be reduced to alcohols by alkali metals in hexamethylphosphoramide.[115] It may be concluded that the sensitivity of the acyl–proline bond is due to the presence of the tertiary amide. This assumption is also supported by further observations in the field of peptide chemistry,[72] i.e., the reduction of tosylglycine piperidide resulted in a mixture of glycine, glycine piperidide, and 2-aminoethanol. Though Rudinger observed peptide bond cleavages during the synthesis of certain analogues of oxytocin,[25] the sodium–liquid ammonia reduction has remained an extensively used method.[18–20]

Some research groups tried to utilize the sensitivity of the acyl–proline bond for the purpose of peptide analysis.[116,117] The application of sodium in large excess led to partial reduction of phenylalanine residues[80,117] and to damage of the N-terminal peptide bond.[117]

IV. Principle of the Removal of Protecting Groups

A. Stoichiometry

Though the amount of sodium consumed in the reduction of simple aliphatic sulfides (eq 1) and disulfides

(eq 2) was exactly determined (2 mol of sodium/mol),[34]

$$R_2S + 2Na + NH_3 = RSNa + RH + NaNH_2 \quad (1)$$

$$R_2S_2 + 2Na = 2RSNa \quad (2)$$

nevertheless, that necessary for the removal of the *N*-benzyloxycarbonyl and *S*-benzyl groups remained uncertain since the appearance and maintenance of the lasting blue color were generally and implicitly considered to be the end point even in the reductions of the simplest amino acid derivatives. This blue end point explicitly means the presence of a surplus of sodium in the reduction mixture. The observation that two cleavage products of the benzyl group, i.e., toluene (**9**) and bibenzyl (**10**) could be detected[2,44] made the situation more complex. The cleavage requires 2 mol of sodium/mol if **9** is formed exclusively (eq 3) but just 1 mol of sodium/mol if **10** alone is produced (eq 4).

$$Bzl\text{-}X + NH_3 + 2Na = 9 + NaNH_2 + NaX \quad (3)$$

$$2Bzl\text{-}X + 2Na = 10 + 2NaX \quad (4)$$

In the field of carbohydrate chemistry, Nayak and Brown made a significant contribution to the knowledge of the cleavage mechanism[118] while studying the removal of *S*-benzyl and *O*-benzylidene groups by sodium in liquid ammonia. In this medium these groups cleaved were converted largely into **9** (only 9% of **10**), while in a mixture of liquid ammonia and 1,2-dimethoxyethane the yield of **10** exceeded 50% in a much slower reaction. In liquid ammonia the *O*-benzylidene required 4 mol of sodium/mol for cleavage, yielding mostly **9**. In the mixed solvent system 16% of **10** was formed and the deprotection was accomplished with less than 4 mol of sodium/mol.

This uncertainty and simplified view of the reaction may explain an inaccuracy found in the most complete and excellent textbook of the peptide chemistry.[25] The stoichiometry of the reduction of the *N*-benzyloxycarbonyl group was described by eq 5. The description

$$BzlO_2CNHR \xrightarrow{2Na/NH_3} 10 + Na_2CO_3 + H_2NR + (9) \quad (5)$$

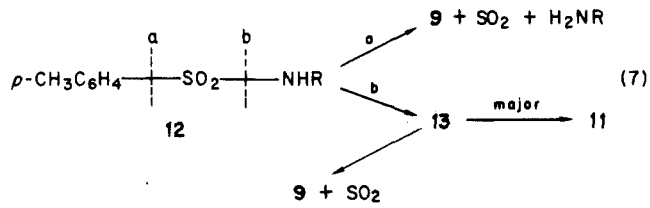
of the transformation takes into account neither possible variation of stoichiometry nor the fact that in the given basic milieu no carbonate ion may form from the carbamate salt[71] in the absence of water (eq 6).

$$^-O_2CNHR \nrightarrow CO_3^{2-} + H_2NR \quad (6)$$

The observed 2 mol of sodium/mol was considered to be much less for the cleavage of *N*-benzyloxycarbonyl group than the calculated amount,[74] especially if the consumption of the inherent proton donors and cleavage products is not neglected.

The mechanism of the reduction of the *N*-tosyl group was much more thoroughly studied. Birch and Smith reported only the formation of thiocresole (**11**)[119] as cleavage product, although the presence of sulfite and sulfate ions in the reaction mixtures had earlier been demonstrated after metal–liquid ammonia reductions of *N*-tosylamino acids and tosyl peptides (**12**).[120] The isolation of the salts of 4-methylbenzenesulfinic acid (**13**) was also later reported.[121] These observations were confirmed by other authors.[121,122] Bajusz and Medzihradszky stated that 2.5–3.5 mol of sodium/mol was required for the deprotection of **12**.[74]

In convincing model experiments Kovacs and Ghatak detected two pathways of *N*-tosyl cleavage.[123] In the major course of the reaction, the cleavage of the C–S bond leads to the formation of SO_2 and **9**, in addition to the amino acid or peptide (eq 7a). The minor path



(7)

is the S–N bond cleavage resulting in **13**, which again undergoes two types of reaction leading primarily to **11** through reduction and to sulfur dioxide and **9** by C–S bond cleavage (eq 7b). It must be mentioned that neither of these reaction schemes takes into account the presence of sodium ion and species derived from ammonia in the reaction mixture.

Another approach to the cleavage of the *N*-tosyl group was given by Rudinger and van den Brink-Zimmermanová.[72] In the case of the reduction of **12** in the form of its ammonium salt (**14**), the formation of 4-methylbenzenesulfinate (**15**) and the carboxylate anion (**16**) requires 2 mol of sodium/mol (eq 8). In to-

$$\underset{14}{CH_3C_6H_4SO_2NHCH_2CO_2NH_4} + 2Na = \underset{15}{CH_3C_6H_4SO_2Na} + NH_3 + \underset{16}{H_2NCH_2CO_2Na} \quad (8)$$

sylamides (**17**) not containing an inherent proton-donating group for every mole of amide reduced, 1 mol of **17** is ionized (eq 9). The irreducibility of the di-

$$\underset{17}{2CH_3C_6H_4SO_2NHCH_3} + 2Na = 15 + H_2NCH_3 + \underset{18}{[CH_3C_6H_4SO_2NCH_3]^-\,^+Na} \quad (9)$$

sodium salt of *N*-tosylglycine lends plausible experimental support to the assumption that the tosylamide anion (e.g., **18**) is resistant to reduction. In accord with this explanation *N*-tosylpiperidine (**19**) can be completely reduced with 2 mol of sodium/mol, the second of which converts ammonia to amide ion (eq 10).

$$\underset{19}{CH_3C_6H_4SO_2N(CH_2)_5} + 2Na + NH_3 = 15 + NaNH_2 + HN(CH_2)_5 \quad (10)$$

Equation 9 may give an explanation to the incomplete reductions in spite of the blue "end point" reached.[87-89,124] The partial deprotection of Tos-Cys-(Bzl)-OH can be understood assuming the faster cleavage of the *S*-benzyl group. The intermediate cleavage product benzyl anion is a stronger base than **18**, so the tosylamino group is ionized and becomes insensitive to further reduction.[72] In accordance with the results of Rudinger and his co-workers,[72,96,121,125,126] Hope stated that the reduction of the *N*-tosyl group resulted in **13** as a main product and occurred with 2 mol of sodium/mol.[127]

The presence of both the inherent proton-donating functional groups and species derived or added affects the course of the reduction by taking part in competitive and consecutive reactions. A good example was provided by Hope.[63] He stated that the reductions of methionine and *S*-benzylhomocysteine consumed 3 and 2 mol of sodium/mol, respectively. After treatment

with 1 mol of sodium/mol, methionine remained unchanged, while a 50% cleavage of the *S*-benzyl group was detected. This observation is obvious, assuming that sodium first affects the *S*-benzyl group. In this case, the ammonium ion forming a salt with the carboxylate ion remains free and reacts with the benzyl anion cleaved to form **9**. In the reduction of methionine, however, at first the ammonium ion reacts with sodium, liberating hydrogen, and then in the resulting proton-free milieu the *S*-methyl group can be cleaved according to eq 1.

Though free amino acids, peptides, and proteins react with sodium in liquid ammonia,[49–51] relatively few investigators recognized the possibility that the terminal and side chain functional groups (OH, $CONH_2$, CO_2H, SH, indolyl, etc.) of peptides contribute to the sodium consumption.[63,72,74,96,127]

Two opposite views arose in the attempt to combat the side reactions accompanying the sodium–liquid ammonia reduction. The addition of sodium amide was suggested by Benisek et al.[117] to eliminate the assumed harmful effects of proton donors present in peptides or formed in the reduction and by Katsoyannis et al.[104,105] to prevent the acyl–proline bond cleavage, though the latter would have known that in some conditions the presence of amide ion causes racemization.[63] On the other hand, precisely the addition of proton donors (e.g., acetamide[68] and urea[69]) was proposed to suppress racemization[63] and the formation of sodium amide[68,69] as well as for completion of the *N*-tosyl group cleavage.[72] The potential dangers accompanying the presence of all these additives were well summarized by Rudinger and van den Brink-Zimmermanová.[72]

B. The End Point and Duration of the Reduction

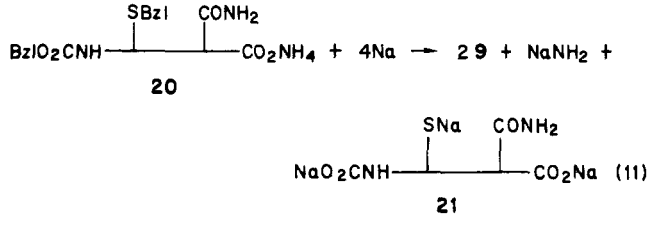
Glancing over the literature of the past half century, it seems to be unambiguous that sodium was always applied in more or less excess during the sodium–liquid ammonia reduction of protecting groups. A lasting light or dark blue color of 5–15 s[108,128] to 1.0–1.5 h[68,129] was generally accepted as the end point of the reductions. The significance of the duration of the treatment was early recognized.[63,87,108,118] During the synthesis of the insulin B chain, Marglin and Merrifield reported that "the stable light blue end point was limited to exactly 15 s in order to prevent the excessive cleavage" of the Thr–Pro bond.[108] Under these conditions the cleavage amounted only to 20–25%, whereas a 60-min treatment led to an 80% cleavage. With the knowledge of the aforementioned facts, it is not surprising that Zahn et al. obtained a product of 0.2–1.0% insulin activity after a treatment of 1.5 h with sodium.[68]

Several types of apparatus were developed for accomplishment of the sodium–liquid ammonia reduction.[87,105,130] In a liquid ammonia apparatus containing a weighed amount of dissolved sodium, the compound to be reduced was added until the blue color just disappeared.[130] Another glass apparatus was mentioned in Section III.[105] Nesvadba and Roth devised a glass apparatus for the reduction, where sodium is dissolved by refluxing ammonia.[87] By rapid cooling of the reaction mixture, the sodium dissolution and the reaction can be frozen. However, also in this case the appearance of the blue color was accepted as the end point of the reductions.

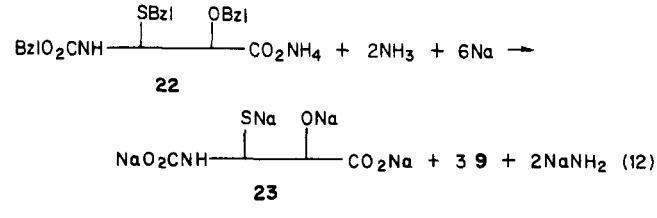
Apart from some analytical applications where sodium was used in very large excess,[116,117] the available data show that in preparative reductions of protected peptides sodium was generally added in up to fivefold excess to the reaction mixture to maintain the lasting blue end point. The excess is calculated on the basis that the reduction of 1 mol of benzyl-type and tosyl protecting groups requires 2 mol of sodium/mol. It will be shown in Section VI that this calculation can be accepted,[29,30] though much more sodium would be necessary for reaching a lasting blue end point, for example, in the reduction of *N*-tosyl derivatives, where **13** is a reactive product of the detosylation.[72,96,123,131]

The following representative examples show the confusion existing in the determination of the stoichiometry and the end point of the reduction. In a recent successful synthesis of the human β-endorphin, a 31-peptide, 0.187 mmol of protected peptide was treated with 28 mmol of sodium for the removal of six benzyl ether, three benzyl ester and six *N*-benzyloxycarbonyl groups.[20] In addition, there were one phenolic hydroxyl and four carboxamide groups as well as one methionine residue in the molecule, all of them capable of consuming sodium. The amount of sodium was considered to represent an ca. tenfold excess. According to the aforementioned calculation, the cleavage of altogether 14 benzyl-type protecting groups requires only 5.61 mmol of sodium, if the role of other competitive reactions is negligible; even so the amount of sodium applied represents only a fivefold excess. Sodium was added in portions over a 45-min period without mentioning the color at the end of the reaction. Though the peptide contains a sensitive Thr–Pro bond and sodium was applied in a large excess, the yield of the highly purified substance was ca. 25%.

During the deprotection of Z-Cys(Bzl)-Gln-OH (**20**), really 4 mol of sodium/mol was added and a blue reaction mixture formed[132] (eq 11). Also, the formation of some **10** may give an explanation for the blue end point though the carboxamide group itself might have been considered capable of consuming 1 additional mol of sodium/mol.



According to the calculation, the deprotection of Z-Cys(Bzl)-Tyr(Bzl)-OH (**22**) requires 6 mol of sodium/mol; however, the application of 3.55 mol of sodium/mol was reported to lead to a blue end point maintained for 20 min[133] (eq 12). In the last two examples at least the completion of the reductions must be assumed, taking into account the formation of some **10**, too. However, no explanation can be found for the lasting blue end points in these reactions; e.g., in the



latter, the free functional groups themselves are able to consume 4 mol of sodium/mol. If in the reduction of **22**, exclusively **10** is the product of cleavage, 3 mol of sodium/mol would have been sufficient for the deprotection; nevertheless, a fourth mol of sodium/mol could have been consumed in the salt formation.

Having reached the lasting blue end point, the excess of sodium and the sodium salts formed in the reduction are generally decomposed by addition of proton donors, e.g., acetic acid,[134-136] ammonium chloride,[20,30,94] ammonium iodide,[47,55] ammonium sulfate,[133] or ion-exchange resins.[93,137] In some cases no additives were provided.[132] There is no doubt that the addition of a proton donor is required at least for the decomposition of the sodium excess and sodium amide in order to prevent undesired side reactions and racemization (Section III). However, it must be remembered that during the discharge of excess sodium by addition of a proton donor, conditions similar to those of Birch reductions exist which certainly affect and accelerate undesired side reactions. The reactivity of this reducing milieu depends on the amount of the sodium excess. Though Marglin found no harmful effect of these proton sources, he could not rule out their possibilities.[115]

Bodanszky and du Vigneaud[138] and later Sakakibara et al.[139] called the attention to another interesting feature of the sodium–liquid ammonia reduction of peptides. In the course of oxytocin syntheses, a significant loss in yield was observed upon scaling up the reduction process. Though no allusion was made, it may be assumed that the longer duration of sodium excess was responsible for this phenomenon.

V. Scope of Applications

In this section representative examples show the scope of applications which are classified according to two points of view, i.e., the field of application and the type of protecting and functional groups.

Apart from the aforementioned successful syntheses of oxytocin[5-9,138,139] and its analogues,[18,69,134,135,140] the vasopressins[14-17,136] and their analogues,[19] the sodium–liquid ammonia reduction of human growth hormone,[129] α-adrenocorticotropin, and insulin derivatives did not work well, and these syntheses were finally accomplished under much milder conditions using new combinations of protecting groups. However, the efficient synthesis of adrenodoxin fragments,[128,141,142] the salmon calcitonin,[100,101] ferrodoxin from *Clostridium pasteurianum*,[143] peptides forming iron–sulfur cluster compounds,[144] poly-L-cysteine,[145] and last but not least human β-endorphin[20] clearly indicates that the sodium–liquid ammonia reduction has remained a valuable tool in the methodological arsenal of peptide chemistry.

The reduction has been extensively used in the field of carbohydrate chemistry, and the work of Nayak and his co-workers[118,146,147] made a significant contribution to the understanding of the mechanism of the cleavage (Section IV.A). In methyl *S*-benzyl-4,6-*O*-benzylidene-2-thio-α-D-altropyranosides the selective cleavage of the benzyl–thio C–S bond vs. the benzylidenedioxy C–O bonds was reported. In these experiments both cleavage products of the benzyl-type groups, i.e., **9** ans **10**, were detected.[118] The ratio of products depends on the solvent. In the presence of 1,2-dimethoxyethane, an inert solvent, the deprotection is slower and the formation of **10** is favored. The selective reduction of *S*- and *O*-benzyl groups by sodium was accomplished in the synthesis of 6-acetamido-1,2,3,4-tetra-*O*-acetyl-6-deoxy-L-idothiapyranose in the presence of an *O*-isopropylidene group.[148] The removal of *S*- and *O*-benzyl and *O*-benzylidene groups was reported in the synthesis of β-amino mercaptans derived from D-altrose,[149] methyl 3-thio-α-D-arabinofuranoside,[150] methyl 2-deoxy-3-mercapto- and 2-mercapto-3-deoxy-α-D-altropyranosides,[151] and β-D-glucopyranose derivatives.[152] The *O*-tosyl group was selectively cleaved by sodium from *O*-isopropylidene-protected α-D-xylo- and α-D-glucofuranose derivatives.[153] The removal of *O*-trityl and *O*-benzyl groups was utilized in the synthesis of sucrose[154] and α-D-glucopyranoside derivatives.[155] Recently the application of the sodium–liquid ammonia reduction was reported in the synthesis of glycopeptides of the bacterial cell wall by Zaoral and his co-workers.[156-158]

Though the nucleosides themselves are sensitive to reduction by sodium,[159] this method was utilized in nucleoside synthesis for debenzylation.[160,161] Of the miscellaneous applications[75,76,78,162-169] the successful accomplishment of the reduction in the synthesis of pantetheine,[165] a cephalosporine analogue,[166] α-lipoic acid,[167] and the cleavage of an *S*-benzyl *S*-oxide derivative[168] can be emphasized. It is interesting to note that **11** was the cleavage product of *N*-(tosylamino)alkyl derivatives of imidazolidines.[169]

A classification of reducible protecting groups according to the different functional groups is presented in Table I. Only those examples are included where the application of sodium–liquid ammonia reduction was actually realized, though there are a number of other protecting groups certainly cleavable with sodium (e.g., all the substituted benzyl type). The *tert*-butyl-type protecting groups are generally considered to be insensitive to reduction;[25,69,99-101] however, the decomposition of *tert*-butyl esters was also observed.[89,190] In a synthesis of oxytocin[191] the hydroxyl group of the tyrosine residue was protected by ethoxycarbonyl group. An unidentified side reaction was detected by amino acid analysis of the end product; therefore, in the modified process this protecting group should have been previously cleaved in liquid ammonia alone. Two research groups studied the reaction of esters with sodium in liquid ammonia in detail.[192,193]

VI. Recent Progress

In recent years we extensively studied the problems accompanying the sodium–liquid ammonia reduction of protected peptides. Searching for potential side reactions of functional groups and rearrangements, we partly selected *N*-*tert*-butoxycarbonyl-protected derivatives in order to simplify following the course of the potential reactions by thin-layer chromatography and/or electrophoresis.[29-31] In general, 10 mmol of protected amino acid or peptide derivative was treated with several 10-mmol amounts of sodium. To the reaction mixture was added sodium in 2–10-mmol portions, depending on the rate of the reaction. The results are summarized in Table II. The third column shows the color of the reaction mixture at the end of the reaction. It should be emphasized that the mixture was a white suspension or a colorless solution in all cases

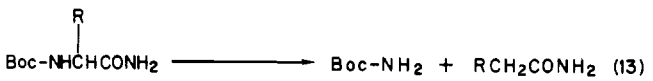
TABLE I. Classification of the Reducible Protecting Groups

protected functional group	protecting group	ref
amino	Tos	3, 120, 121
	Z	2, 42
	(allyloxy)carbonyl	170
	[[2-(chloromethyl)-2-propyl]oxy]carbonyl	171
	[[2-(bromomethyl)-2-propyl]oxy]carbonyl	171
	benzylthiocarbonyl	172
	4,5-diphenyl-4-oxazolin-2-one[a]	173
carboxyl	Bzl	20, 55, 132, 174–176
	4-picolyl	177
	2-(methylthio)ethyl	178, 179
hydroxyl	Bzl	20, 133, 146, 153, 160, 180, 181
	benzoyl	182
	pivaloyl	182
	benzylidene	147
	Tos	152
	Trt	153, 154
mercapto	Bzl	1, 36, 76
	Bzl-*S*-oxide → SO_2H	168
	4-methoxy-Bzl	139
	4,4′-dimethoxydiphenylmethyl	183
	Trt	184
	diphenylmethyl	184
	ethylcarbamoyl	185
N-imidazole	Bzl	3, 75, 186
N-guanidino	Tos	187–189

[a] The protected amino function is in the ring.

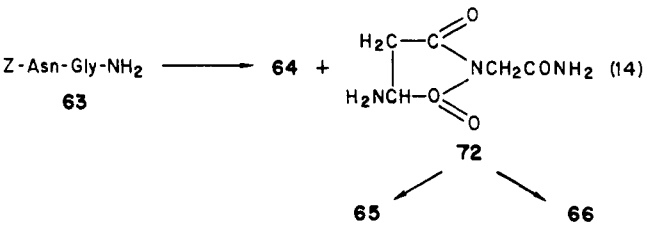
when a protecting group was completely removed and even in most cases when sodium was added in an amount larger than necessary for neutralization of the proton-donating functional groups. *This observation essentially contrasts with all earlier customs and views postulating some definitely blue end point of the deprotections.* Aside from local excess, therefore, in these reactions there was no sodium surplus at all.

As shown in Table II, several new side reactions were observed. (a) From the reaction of **28**, **29**[194] was isolated. We did not observe an analogous reaction of other *N*-(benzyloxycarbonyl)amino acid amides. (b) During the reduction of primary carboxamide derivatives (**30**, **33**, **35**, **39**, **42**, **45**, **57**, and **60**), a partial reduction to alcohol derivatives (**32**, **34**, **38**, **40**, **43**, **46**, **58**, and **61**, respectively) occurred in accord with Chablay's earlier observation,[195,196] which escaped the attention of peptide chemists nearly for 70 years. (c) In addition to the aforementioned alcohol derivatives formed during the reduction of α-(*tert*-butoxycarbonyl)amino acid amides (**42**, **45**, **57**, and **60**), the splitting of the N–C_α bond was observed with the formation of **36** and the appropriate deaminated product (**44**, **47**, **59**, and **62**, respectively) (eq 13). In **60** no cleavage of N–C_ϵ could be detected.



starting material	R	product
42	Bzl	44
45	4-hydroxy-Bzl	47
57	4-aminobutyl	59
60	4-(Boc-amino)butyl	62

(d) During the treatment of **55** the benzene ring of the indolylmethyl side chain partially saturated to form **56**.[79] (e) The reduction of **63** led to partial deamidation and transpeptidation via the proposed intermediate, L-*N*-(aminosuccinyl)glycinamide (**72**) (eq 14). The



extent of this rearrangement increased with the amount of sodium used and was significant only in the Asn-Gly sequence.

VII. Conclusions

With the knowledge of the earlier and our recent results, several conclusions can be drawn. (a) In accord with the earlier observation of Bajusz and Medzihradszky,[74] we confirmed that the removal of the *N*-benzyloxycarbonyl group requires approximately 2 mol of sodium/mol,[29,30] though the exact amount of sodium can be only experimentally determined in every case because of the competitive reactions and of the individuality of peptides containing other cleavable groups (e.g., *S*-methyl in methionine) and different proton-donating functional groups (CO_2H, $CONH_2$, OH, indolyl, imidazolyl). Consequently, it is quite unambiguous that the real end point, i.e., the completion of the protecting group removal, should be clearly distinguished from the appearance and maintenance of the blue color of the reduction mixture. Apart from some special cases[87–89,124] (e.g., eq 9), no blue end point is needed for the complete deprotection! The rate of the *N*-benzyloxycarbonyl cleavage significantly exceeds that of the deprotonation to ammonia of ammonium ion, stemming from salt formation of the carboxyl group in liquid ammonia.

(b) Essentially the same consideration is true for the cleavage of the *N*-tosyl group in agreement with the earlier observations.[72,124] The reduction of *N*-tosyl and *S*-benzyl groups takes precedence over the deprotonation of ammonium ion in reaction with sodium.[124,131] As the reduction of the *S*-methyl group is slower than the deprotonation of ammonium ion,[62] the deprotection of peptides containing methionine residue(s) can be accomplished if sodium is not added in an excess.

(c) Similarly to the cleavage of the *S*-methyl group, the rate of salt formation of the carboxamide group is slower by approximately 1–2 orders of magnitude than that of the neutralization of the ammonium ion.[29–31] The salt formation of the carboxamide group is competitive with its reduction to the alcohol derivative.[31] Both of these reactions can be prevented if sodium is not used in an excess during the deprotections.

(d) The treatments of homoserine[31] and tyrosine[197] derivatives with sodium showed that the rate of the salt formations of both the side-chain aliphatic and phenolic hydroxyl groups is very slow as compared to that of the reduction of the protecting groups and the salt formation of other functional groups including even the carboxamide group.

(e) *The optimal amount of sodium* necessary only for

TABLE II. Results of Na–Liquid NH_3 Reduction of Model Compounds[a]

starting material	mol of Na/mol	color of rctn mixture[b]	products: isolated	products: detected (estimated %)[c]
Z-Cha (**24**)	1.5	W	**24** (15%)	Cha
	1.9	W	**24** (1%)	Cha
	2	B		Cha, **24** (traces)
Z-Ala-OH (**25**)	2	W	**25** (1%)	Ala
	2.25	B		Ala
Z-Gly-NH_2 (**26**)	1	W	**26** (40%)	
	2	W	**26** (2%)	H-Gly-NH_2 (**27**), Gly (traces)
	2.2	B		
H-Pro-NH_2 (**4**)	1 + 1	W + B		**3**, **4**, **5**, + unidentified products
Z-Pro-NH_2 (**28**)	1	W	Pro hydantoin[d] (**29**, 17%)	**4**, **28**
	2	FB		**4**, **28**, **29** (10%)
Boc-Asn-OH (**30**)	2	SW	lactone of Boc-Hse-OH (**31**, 12.6%)	**30** (70%), Boc-Hse-OH (**32**, 30%)
	3	B	**30** (54%), **32**[e] (25%)	
Boc-Gln-OH (**33**)	1	W		**33** (100%)
	2	SW	**33** (63%), 2-(Boc-amino)-5-hydroxyvaleric acid[e] (**34**, 9.5%)	**33** (70%), **34** (30%)
Boc-Asp-NH_2 (**35**)	1	W		**35**, Boc-NH_2 (**36**, 1%) isoasparagine (**37**, traces)
	2	SW	**35** (43%), **36** (8.7%) 3-(Boc-amino)-4-hydroxybutyric acid[e] (**38**, 2%)	**36** (20%), **38** (traces) **38** (25%)
Boc-Gly-NH_2 (**39**)	1	W		**39**, Boc-ethanolamine (**40**) ethanolamine (**41**), **27**, **36**
	2	B	**39** (17%), **40** (34%)	**27**, **36**, **41**
Boc-Phe-NH_2 (**42**)	1, 2	W, B	**42** (63%), Boc-phenylalaninol (**43**, 2%), 3-phenylpropionamide (**44**, 6%)	
Boc-Tyr-NH_2 (**45**)	2, 3	W, B	Boc-tyrosinol (**46**, 1%), 3-(4-hydroxyphenyl) propionamide (**47**, 12%)	**36**, **45**, H-Tyr-NH_2 (**48**)
Boc-Gly-OH (**49**)[f]	1 + 1	W + SW	**49** (92.5%)	
Z-Asn-OH (**50**)	2	W		**50** (2%), Asn, Hse-lactone (**51**, 1%)
	3	B		**50**, Asn, unidentified products
Z-Tyr-NH_2 (**52**)	2	W	**52** (4%)	**48**
	3	B		**48**
Boc-Arg(Tos)-OH (**53**)[g]	2-5	W-SW		Boc-Arg-OH (**54**), unidentified product
Boc-Trp-OH (**55**)	1 + 1	W	**55**, dihydro derivative (**56**, 20%)[h]	**55**, **56**
Boc-Lys-NH_2 (**57**)[i]	1 + 1	W + B		**57**, Boc-lysinol (**58**), **36**, 6-aminocapronamide (**59**)
Boc-Lys(Boc)-NH_2 (**60**)	1 + 1	W + B		**60**, Boc_2-lysinol (**61**), **36**, 6-(Boc-amino)-capronamide (**62**)
Z-Asn-Gly-NH_2 (**63**)	2	W		**63** (2%), H-Asn-Gly-NH_2 (**64**), H-Asp-Gly-NH_2 (**65**) + H-β-Asp-Gly-NH_2 (**66**) (1-5%)
	3	SW		**63** (traces), **64**, **65** + **66** (10–20%), unidentified products
Z-Asn-Phe-NH_2 (**67**)	2	W	**67** (7%)	H-Asn-Phe-NH_2 (**68**)
	3	SW		**67** (traces), **68**, two unidentified products (a few percent)
Z-Gln-Gly-NH_2 (**69**)	2	W	69 2%	H-Gln-Gly-NH_2 (**70**) Glp-Gly-NH_2 (**71**), three unidentified products (1%)
	3	B		**70**, **71**, four unidentified products (a few percent)

[a] A part of the table is taken from ref 30 with the kind permission of Munksgaard Int. Publ. Ltd. [b] W, white suspension; B, lasting blue mixture; SW, slowly whitening suspension; FB, faint blue suspension. [c] By thin-layer chromatography and/or thin-layer electrophoresis. [d] Reference 194. [e] Isolated as dicyclohexylammonium salt. [f] Reference 201. [g] Reference 131. [h] Reference 79. [i] Used as its hemioxalate salt.

the (nearly) complete reduction of the protected peptides and compounds of other types *should be always experimentally determined* in order to prevent or to minimize the formation of undesired byproducts. In some cases acceptance of an incomplete deprotection may be more advantageous as the purification of the end product is easier in the presence of some percent incompletely deprotected products than in that of the "overreduced" by products. The application of sodium in optimal amount for the removal of the protecting groups does not lead to a blue end point contrary to all earlier views.

Our studies in the field of sodium–liquid ammonia reductions resulted in three practical applications. (a) The first is the optimization of the reduction in the synthesis of biologically active peptides.[198]

(b) Recognizing the competitiveness of the salt formation and the reduction of the carboxamide group to an alcohol derivative,[29-31,195,196] we developed two theoretically equivalent methods for the complete conversion of the carboxamide group to an alcohol derivative. It must be stressed that because of this competitiveness no complete reduction can be reached, no matter how large is the sodium excess. The carboxamidate salt in the sodium–liquid ammonia reduction was either cyclically decomposed by addition of ammonium chloride to the reaction mixture or continuously in steady state in the presence of methanol in

order to prepare the carboxamide derivative for the same competitive reactions. The reduction is generally complete in 10–15 cycles or in the presence of 30 mol of methanol/mol. For example, by use of this method, **30** was transformed to **32**, isolated as the dicyclohexylammonium salt[29–31,199,200] with an 80% yield. No racemization was detected.

(c) N-Alkylation and aralkylation of the disodium salt of *N*-(*tert*-butoxycarbonyl)amino acids were accomplished with moderate yields.[201]

Our work also demonstrates the value of investigation of byproducts in determining the scope and limitation of old synthetic methods. The knowledge of the mechanism and kinetics of their formation may help not only in the elimination or minimization of these side reactions but also in their utilization in the development of new synthetic methods. This review was prepared in the hope that it may contribute to newer applications of the sodium–liquid ammonia reduction in peptide chemistry as well as in other fields.

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unsuccessful by column chromatography. It should be mentioned that in this experiment all the Na applied was in excess relative to the amount that would have been necessary for the removal of any protecting groups generally used (see also Section IV).
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