87. A New Reagent for the Cleavage of NPS-Amino Protecting Groups in Peptide Synthesis

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Summary. The value of several reagents capable of removing quantitatively the α-amino protecting group of NPS-aminoacyl- or peptidyl-derivatives is assessed, by comparison with the reagents currently used, *i.e.* hydrogen chloride, RS^--nucleophiles or alkyl thioamides, especially with regard to reaction specificity and end-product solubility. New reagents proposed, 3-nitro-4-mercapto-benzoic acid, and its methyl ester, appear to optimize the numerous, and often competing, requirements. These were probed, under conditions suitable for both conventional and solid-phase methods, on a series of model compounds, including diversely protected amino acids and short peptides, as well as for the actual synthesis of a triacontapeptide by the stepwise, solid-phase approach.

Abbreviations used for amino acids and their protecting substituents concur with Rules of the IUPAC-IUB Commission on Biochemical Nomenclature, *cf.* J. biol. Chem. *247*, 977 (1972), and in addition those taken from the Int. J. Peptide & Protein Research *7*, 91 (1975).
Boc-: *t*-butyloxycarbonyl-; Ppoc-: phenylisopropyloxycarbonyl-; Bpoc-: biphenylisopropyloxycarbonyl-; NPS-: *o*-nitrophenylsulfenyl-; (S)-Ec-: (S)-ethylcarbamoyl-; DMF: dimethylformamide; DMSO: dimethylsulfoxide; NEt_3: triethylamine; DTT: *Cleland* reagent, dithiothreitol; DTNB: *Ellman* reagent, 5,5′-dithio-bis-(2-nitrobenzoic acid); Tos-: tosyl-; -OBzl: benzyl ester/ether; O(*t*-Bu): *t*-butyl ester/ether; -OMe: methyl ester; Dcha: dicyclohexylamine; THF: tetrahydrofuran; THP: tetrahydropyran; NTBA: 3-nitro-4-thiol-benzoic acid (3-nitro-4-mercapto-benzoic acid).

Introduction. – Among the numerous protecting groups utilized for blocking the N(α) function of amino acids during the elaboration of peptides, NPS- has been much favoured in recent years. In essence, the rationale for the precedence of NPS- over Boc- [1], or of the closely related groups, such as Bpoc- [2] or Ppoc- [3] characterized by an increased sensitivity toward acids, is to have the Boc- group available for the protection of individual amino acid side chains. In addition, the NPS- derivatives are easy to synthesize and the NPS- moiety is, conversely, readily split off by dilute acids, such as 0.04 N HCl in dioxane.

As often observed, these improved qualities are accompanied by some disadvantages. Thus, the NPS- derivatives are not stable in their free anionic form, neither in solution nor in the solid state, and must be isolated and stored as carboxylates, *e.g.* as Dcha salts; the acid form must therefore be regenerated prior to each condensation step. Actually, when used in combination within an single peptide, the intrinsic labilities of the N(α) NPS- and the ω Boc- or O(*t*-Bu) groups do not diverge sufficiently to permit the exclusive acidolysis of the first. Another danger, that so far effectively precluded general use of NPS- derivatives in the stepwise solid-phase synthesis, arises

from its inherent reactivity toward the indole ring of Trp, in the presence of hydrogen chloride in polar, or slightly polar, organic solvents. Under these conditions, the released NPS- group is partly reconverted into *o*-nitrophenylsulfenyl chloride, which is then covalently attached to the Trp-indole as an arylsulfide. Hence, protection of Trp by formylation of the indol-N atom has been advocated by some workers [4].

A further risk encounted in Trp containing peptides, relates to the extreme sensitivity of this amino acid to oxidants, even atmospheric oxygen, when acidic media have to be used. Before the advent of the NPS-group, the addition of alkyl mono- or dithiols, such as 2-mercaptoethanol or of DTT (*Cleland's* reagent) was a common precautionary measure; it is evidently no longer feasible with the NPS-protected derivatives.

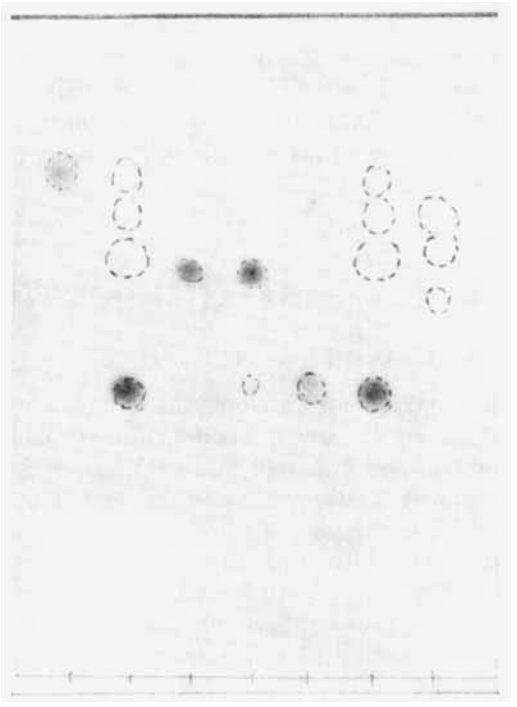
Accordingly, one obvious way to circumvent these drawbacks, while taking full advantage of the beneficial attributes of the NPS-group, was to find another mechanism for its removal, entirely avoiding acidolysis. Valuable preliminary exploration was provided by *Kessler & Iselin* [5] and later by *Wünsch et al.* [6], who probed series of organic and inorganic reagents which operate NPS-group cleavage by direct nucleophilic attack on the electrophilic sulfur of the sulfenylamide linkage. Thioacetamide or thiourea, on the one hand, and thioglycolic acid, on the other, were proposed as being the most satisfactory. However, these reagents and also thiophenol appear to be insufficiently specific, and often too reactive in the long run and by repetitive exposures, for not becoming hazardous for a number of potentially reactive sites along a growing peptide. Although negligible for short peptides, the risk of adverse effects becomes very crucial in the elaboration of long sequences comparable with the wide diversity of residues displayed by most natural, genetically coded, polypeptides in hormones, animal toxins or enzymes. In this respect, the somewhat less reactive thiolytic nucleophiles, such as the substituted aryl thiols, *e.g. o*-nitrothiophenol, are to be preferred, in spite of their slower reaction velocities [7].

Nevertheless, it occurred to us that a major difficulty in the application of these last reagents in routine peptide synthesis is due to the precipitation of insoluble, or only sparingly soluble, disulfides arising in course of NPS- cleavage. This is particularly frustrating for the stepwise solid-phase approach, since the insoluble material tends to cling tenaciously to the resin and clog the beads. Investigations have therefore been carried out in order to evaluate other, and hopefully better, cleaving reagents for the NPS-group, under conditions consistent with either the conventional, or the solid-phase methods, and to assess their value relative to that of the reagents now mostly used. The emphasis has been directed towards a) specificity of reaction and b) solubility of all end- or side-products. As an ancillary result, the latter issue led us to foresee a substitute for NPS-, which would render the formation of insoluble disulfides practically impossible.

Results. – Unless Trp was present, the standard acidolytic cleavage of the NPS-protecting group was still carried out, without apparent structural rearrangement, with very dilute hydrogen chloride. Under these circumstances, we evaluated the extent of irreversible, intramolecular NPS-group transfer that occurs when Trp is being incorporated in a peptide, or is already part of a preformed sequence. Each

time, N(α)-NPS-TrpO$^-$ Dcha$^+$ was submitted to deprotection by an exposure of 30 min at 20° to 0.04 N HCl in anhydrous, peroxide free dioxane, and the end-products were examined. After separation by TLC., under UV. light or after ninhydrin spray, two major spots were observed, apart from traces of unreacted material (fig. 1). The spot corresponding to the transalkylated Trp is about the same size and intensity as that of the regenerated, free Trp, *i.l.* the ratio of alkylated-indole to free Trp is roughly 1:1, indicating that even such mild treatment must be ruled out.

To locate the alkyl group in the by-product, larger quantities of N(α)-NPS-TrpO$^-$ Dcha$^+$ were treated by HCl/dioxane, the reaction products were separated by column chromatography and crystallized [8]. Evidence was thus provided for a free carboxylic group, while the intense reaction observed with ninhydrin is a proof, in semi-quantitative terms, of N(α) deblocking. The chemical structure of the compound was ultimately ascertained by a combination of data, from UV.-vis., IR. and NMR. spectra. This last, in conjunction with the corresponding spectra of unsubstituted Trp, indicated the disappearance of the signal (and spin couplings) of the C(2)-proton on the indole heterocycle. The fragmentation pattern revealed by mass spectrometry was entirely consistent with this observation. On the other hand, the =N–H signal was unambigously identified in the IR. spectra. All data support the existence of a thioether to the C(2)-position of the indole ring, in accordance with the previously postulated structure (see Fig. 1).

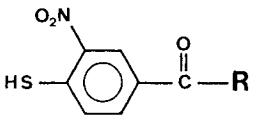


a b c d e f g

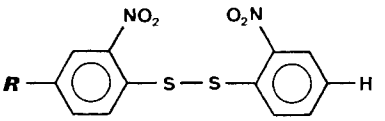
Fig. 1. *Comparative cleavage of NPS-TrpO$^-$Dcha$^+$ by HCl/dioxane and by NTBA.* Coloured spots indicate ninhydrin positive compounds; dotted spots UV-fluorescent, but ninhydrin negative substances. TLC on Silicagel F 254 with BuOH/AcOH/H_2O 4:1:1. Migration towards top of figure. a: N(α)-NPS-TrpO$^-$Dcha$^+$ standard, b: N(α)-NPS-TrpO$^-$Dcha$^+$ + NTBA after 5 min., c: Trp 2-NPS-indole standard, d: N(α)-NPS-TrpO$^-$Dcha$^+$ + HCl/dioxane after 5 min., e: Trp standard, f: N(α)-NPS-TrpO$^-$Dcha$^+$ + NTBA after 24 hrs., g: NTBA standard (oxidation products formed during TLC).

Assessment of new, simple aliphatic and inorganic reagents. A preliminary search for milder reagents, capable of effecting serial NPS- group deblockings more selectively than the aforementioned anionic nucleophiles, focussed attention on three, so far untested, compounds fit for solid-phase technology and for the synthesis of Trp-containing peptides; the reaction products they generate during cleavage are all soluble. Thus selenurea and sodium hydroselenide both exert a complete cleavage, and also have the advantage over their corresponding, admittedly less reactive sulfur counterparts, inasmuch that excess reagent can be readily separated by air oxidation, followed by precipitation of free Se. Among the organic mercaptans, *dl*-thiomalic acid (*dl*-mercapto succinic acid), which would lead predictably to more polar and more soluble side-products in dioxane or DMF, proved also to be effective by cleaving NPS-amino acid derivatives and N(α) NPS- peptides quantitatively at room temperature. The reaction develops at a somewhat slower pace, yet cleavage reaches completion within 2 h, and *dl*-thiomalic acid is advantageous by rendering admixture with acetic acid superfluous. Actually, all three new reagents permit a selective and quantitative deblocking of any N(α) NPS- groups substituted in amino acids and their ω-protected derivatives, or peptides, without affecting the most sensitive side chain protecting groups, *i.e.* Boc-N(ε)-Lys, Glu(O-*t*Bu) or Asp(O-*t*Bu), while preserving the Trp indole ring intact.

New aromatic thiols as NPS- cleaving reagents. The use of thioacetamid, as recommended by *Kessler* & *Iselin* [5] and *o*-nitrothiophenol, as suggested by *Zahn* [7], were found to expel NPS- from N(α)-NPS-PheO$^-$ Dcha$^+$ quantitatively in 3 min at room temperature, with acetic acid as solvent. No alterations could be detected during the routine elaboration of oligopeptides with 15 residues, neither on the amino acid side chains, nor in the protecting groups themselves, nor on the peptide-resin linkages. However, the disturbance caused by the formation of insoluble side-products, namely bis-(*o*-nitrophenyl)-disulfide (**4**) and, using thioacetamid, bis-(*o*-nitrophenyl)-trisulfide as in [9], could never be overcome, despite numerous essays in a variety of solvents.



1 R = OH **2** R = OCH_3



3 R = COOH **4** R = H

We therefore devised a thiolytic reagent, taking *o*-nitrothiophenol as model, by introducing into the aromatic ring a third substituent which, besides being strongly polar, would enhance the maximal orientation effect imparted to the thiol-group by the adjacent nitro-group. As this last substituent is *ortho* relative to the thiol-group, the best choice for the new substituent was a carboxylic, or a methyl carboxylate group, situated in *para* position. This new reagent, 3-nitro-4-mercapto-benzoic acid (**1**) (NTBA), and its methyl ester (**2**) (NTBA-OMe), have both been synthesized, see exper. part. The aryl thiol **1** reacts with *o*-nitrophenylsulfenyl chloride to form the mixed disulfide **3**, which is *soluble*, as opposed to **4** above; **3** is also obtained when any N(α)-NPS-amino acid, as Dcha salt, is exposed to NTBA in polar, anhydrous organic solvents, at room temperature. The thiol sulfur, of oxidation state -2 in NTBA,

reacts readily with the sulfenylamide sulfur at oxidation level 0, by an oxidation-reduction process involving both S atoms. The free amino group is regenerated, while the mixed disulfide is formed, and no insoluble product can actually be detected during this reaction or afterwards. Theoretically, however, the appearance of minute amounts of bis-(*o*-nitrophenyl)-disulfide (**4**) was predictable, as a result of an oxidation-reduction exchange between excess NTBA and the mixed disulfide **3**, thus releasing equimolar quantities of *o*-nitrothiophenol which, in turn, can generate the *insoluble* symmetrical disulfide **4**. As this was never observed, even after a prolonged period of application, it appears that the maximum concentration of **4** remains well below the solubility limit, and that the equilibrium thermodynamic constants for these sulfide-disulfide exchange favor **3**. NTBA-OMe behaved similarly. NTBA and NTBA-OMe were prepared by reduction of their symmetrical disulfides; crystallized four times, both were in the form of pale yellow, almost odorless, crystals. In view of the lack of reference data for these two compounds, their individual constitutions have been carefully checked and extensively reported. Elemental analysis[1]) was performed on the immediate disulfide precursor. The structures were based mainly on IR. and NMR. spectra, and substantiated by mass spectrometry of NTBA-OMe in vapour form (*cf.* exper. part).

The best operating conditions for the cleavage of N(α)-NPS-protecting groups by NTBA or by NTBA-OMe differ slightly according to the procedure adopted for the entire peptide synthesis. For the conventional, single-phase technique, NTBA in DMF is most convenient, though other solvents, such as THF or THP, are also suitable. With a 5 molar excess of NTBA, the deblocking of N(α)-NPS-amino acids in the form of Dcha salts, or of N(α)-NPS-peptides, reaches completion in less than 1 min at room temperature. For the solid-phase approach, NTBA-OMe is preferable, mainly because it precludes any potential interaction with the aminoacyl-resin ester linkages, and may improve surface contact with the polystyrene matrix. Though dioxane was used initially, methylene chloride, or methylene chloride/dioxane, proved to be more satisfactory, presumably because CH_2Cl_2 allows for maximum swelling of the resin beads. Hence, the optimal conditions require a 5 to 10 fold molar excess of NTBA-OMe, dissolved in methylene chloride. A reaction time of 4 h is sufficient for completion of the reaction in the least favorable case. The optimal duration may be shortened, depending on the nature of the N-terminal amino acid itself or its side chain protecting group, and upon the length and composition of the peptide sequence concerned.

It was observed with both reagents that small quantities of Dcha acetate do not hinder the cleavage but, on the contrary, facilitate it noticeably. Catalytic amounts of NEt_3 acetate, as well as acetic acid, accelerate the cleavage. The two outstanding attributes of NTBA and of NTBA-OMe, efficiency (completeness *vs.* time) and reaction specificity, are best illustrated in the two following series of experiments.

In the first resin-bound NPS-GlyO-resin was deprotected, in parallel runs, a) by *o*-nitrothiophenol, b) by NTBA and c) by NTBA-OMe. For the sake of comparison, an equal molar excess and a unique solvent were used throughout; they were arbitrarily chosen so as to optimize the *o*-nitrothiophenol reaction. The results are depicted in fig. 2. The profiles of 2A show that only 81% of the NPS-group is split off in 24 h

[1]) Carried ont by Dr. *K. Eder*, Geneva.

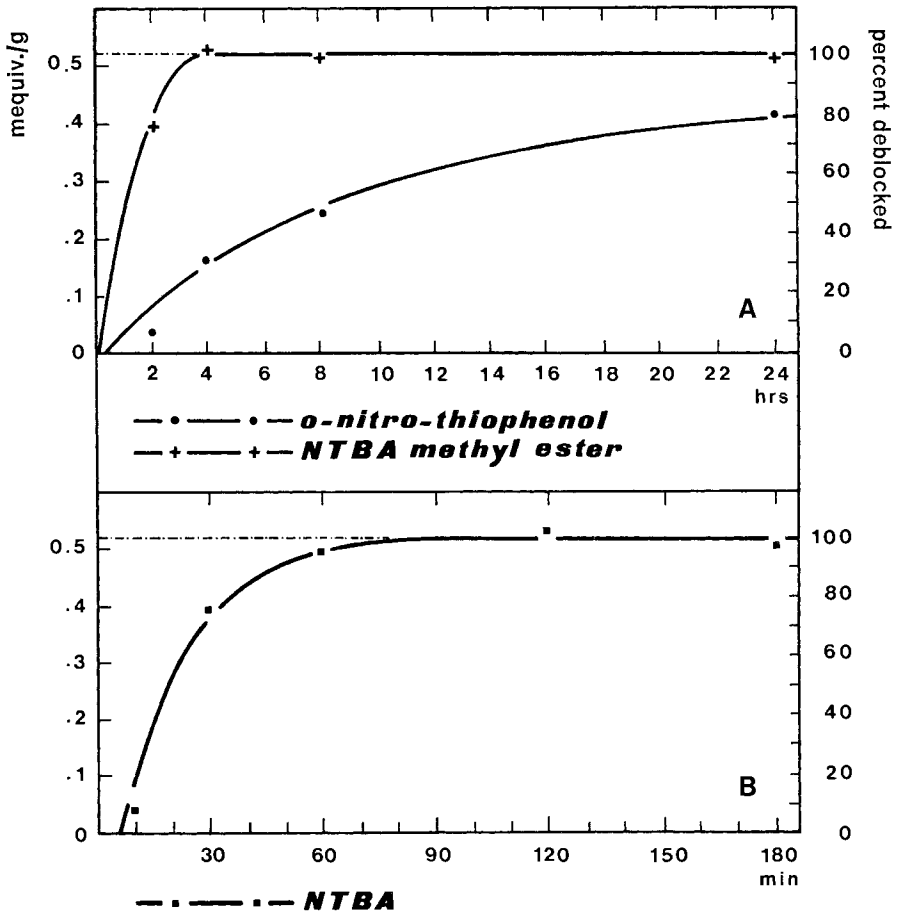


Fig. 2. *Comparative rates and efficiency of NPS-cleavage on solid support by* o-*nitro-thiophenol, NTBA and by NTBA-OMe. 2A: reaction profiles for* o-*nitro-thiophenol* vs *NTBA-OMe; 2B: reaction profile for NTBA at expanded (8×) time scale.* Substrate: NPS-GlyO-resin at 0.55 ± 0.03 meq. per g. Reagents in 7 × molar excess; solvent: dioxane; temperature: 20°. These conditions are optimal for *o*-nitro-thiophenol cleavage, but suboptimal for NTBA and NTBA-OMe

by *o*-nitrothiophenol, despite conditions ideally suited for this particular reagent, whereas NTBA-OMe, in a less than optimal medium, accomplishes total cleavage in 4 h. On the same substrate, also attached to the insoluble resin commonly used in solid-phase synthesis, the free acid, NTBA, is even slightly more active, as seen in 2B, showing 8 fold expansion on the time scale. Even with larger substrates, NTBA-OMe and NTBA achieve complete cleavage in a remarkably short time, as compared to that of thiophenol or *o*-nitrothiophenol, operating under the same conditions.

The second series of experiments comprised two kinds of substrates, those with N(α)-protecting groups other than NPS-, *i.e.* Z-, Boc- and Bpoc-, or with O(*t*-Bu) blocking the C(α) carboxylic function, and those having their side chain functional groups protected by appropriate substituents, *i.e.* -OBzl esters and ethers, (S)-Ec-

group, *etc.* The duration of their reaction with NTBA, NTBA-OMe resp., was 24 h, a period that surpasses the maximal duration of NPS-cleavage by a factor of 5 when working with the insoluble resin support, and by a factor of several hundreds in the case of readily soluble substrates. Under the conditions of these essays, an N(α)-NPS-group would have been expelled in about 1 min. The results are summarized in Table 1. Nowhere could functional group deprotection be detected; free Trp, as well as N(α)-Boc-Trp, were also integrally recovered.

Table 1. *Sensitivity of various protecting groups towards NPS-cleaving reagent.* 10 μmoles of amino acid derivative dissolved in minimum amt. of dioxane or methylene chloride + 10 mol. equiv. of NTBA in 0.5 ml dioxane, temp. 20°, samples tested by TLC

Amino acid derivative	Exposure in h	Remarks
Cys(Ec)	24	Ec-resistant
Bpoc-IleDcha	24	Bpoc-resistant
Bpoc-His(Boc)	24	Bpoc + Boc-resistant
Bpoc-His(Tos)	24	Bpoc + Tos-resistant
Bpoc-Tyr	24	Bpoc-resistant
NPS-Asp$_\beta$(O*t*Bu)	96	Asp$_\beta$(O*t*Bu) resistant
Boc-Phe	96	Boc-resistant
Phe-O*t*Bu	96	-O*t*Bu resistant
NPS-Arg(Boc)$_2$	24	Arg(Boc)$_2$ resistant
Tyr(OBzl)	24	intact
Thr(OBzl)	24	intact
Ser(OBzl)	24	intact
NPS-Tyr(O*t*Bu)Dcha	24	Tyr(O*t*Bu) resistant
NPS-Thr(O*t*Bu)Dcha	24	Thr(O*t*Bu) resistant
NPS-Ser(O*t*Bu)Dcha	24	Ser(O*t*Bu) resistant
NPS-Asn Dcha	24	Asn intact
NPS-Cys(Bzl)	24	Cys(Bzl) resistant

Improved N(α)-amino protecting group. The choice of NTBA or NTBA-OMe for cleaving NPS-protecting groups during peptide synthesis is subject to one restriction, hardly effective in practice, which requires theoretically that small amounts of insoluble bis-(*o*-nitrophenyl)-disulfide be formed in the course of the reaction. In keeping with the rationale of this study, we thought it desirable to investigate an analogue of NPS- that would render this outcome virtually impossible. Hence, this analogue must have a carboxylic, or a methyl carboxylate, group in *para*-position, relative to the sulfur atom. This reagent, methyl 3-nitro-4-chlorosulfenyl-benzoate, has been synthesized *via* the symmetrical disulfide, which was obtained from 3-nitro-4-chlorobenzoic acid by the application of the method described by *Havlik & Kharasch* [10]. After the esterification of both carboxylic groups by diazomethane, direct chlorination in the presence of iodine furnished methyl 3-nitro-4-chlorosulfenyl-benzoate, which was isolated and purified and was ultimately obtained in the form of pale yellow crystals. Dissolved in DMSO, it reacts readily with free amino acids, in a slight molar excess of NEt_3, to yield quantitatively the corresponding N(α)-arylsulfenyl-amino acid derivatives. Amino acids protected by this new substituent have, in turn, been isolated chromatographically and characterized; their N(α)-protecting group can be readily split off by reaction with NTBA or NTBA-OMe in dioxane. Its systematic use in routine peptide synthesis by the solid-phase approach is in course of evaluation.

Discussion. – The two aryl thiols proposed as an alternative to reagents customatily applied for the cleavage of the N(α)-NPS-protecting group proved to be more efficient and selective, at the same time preventing the formation of impeding insoluble, or sparsely soluble, end- or side-products. NTBA appears to be ideally suited to NPS-deprotection in peptide synthesis by way of fragment condensation, whereas NTBA-OMe is particularly adapted to the operational requirements of the solid-phase procedure. An NPS-transfer, such as any Trp derivative undergoes during acidolysis, is entirely avoided. Furthermore, the new reagents eliminate the need for alkyl mercaptans in order to preserve Trp residues from oxidative degradation and preclude the adverse effects ordinarily associated with the need for a large excess of thiophenols or thioacetamide to effect complete thiolysis of the NPS-group.

The new reagents offer the additional advantage of eliminating the appearance of insoluble disulfides, which normally arise on thiolysis with thiourea, thioacetamid, thiophenol and even *o*-nitrothiophenol. Complete solubility of all reaction products or side-products, save the resin-bound peptide itself, is indeed of the utmost importance for a successful stepwise synthesis on solid-phase, once the peptide elongation exceeds about twelve residues.

Moreover, the high specificity attained by NTBA and NTBA-OMe in the cleavage of the NPS-substituent makes a number of other protecting groups, previously applied to N(α)-protection, available for side chain protection without risk of uncontrolled deblocking. This makes the choice of an appropiate combination of temporary *vs* long-lasting protecting groups more accessible and may even put within reach the much desired possibility of removing all side chain protecting groups in one single operation after completion of the synthesis, *e.g.* by liquid HF.

It has been argued in the literature that the removal, before each coupling step, of Dcha linked to NPS-protected amino acids, is a time-consuming process, and that severe caution has to be exercised in the acid treatment leading to its liberation. In our work, this did not cause difficulty; Dcha was routinely eliminated from stock NPS-amino acids by titrating their aqueous solution to pH 3 with 0.5 N H_2SO_4, followed by immediate extraction with ether or ethyl acetate and evaporation. Once dried, N(α)-NPS-amino acids were found to be stable for 24 h at 0°.

The solubilisation effect contributed by NTBA-OMe can be further enhanced, if the N(α)-NPS-group is replaced by methyl 3-nitro-4-sulfenyl-benzoate. The major attribution of this NPS-analogue is to by-pass the formation of insoluble disulfides and to lead to dimethyl 4,4'-dithio-bis(3-nitro-benzoate), a compound freely soluble in dioxane or DMF. Derivatives of several amino acids so obtained have been successfully isolated, crystallized and ultimately submitted to cleavage by NTBA-OMe, which proceeded swiftly and selectively, and gave no insoluble end-products. More experience is needed, however, especially in routine stepwise peptide synthesis on solid-phase, before the validity of this new amino-protecting group can be fully ascertained.

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Table 2. *Comparative survey of the efficiency of NPS-cleaving reagents*

No.	Blocked amino acid	Quantity	Cleaving reagent	Experimental conditions	Time	Deblocked
1	NPS-Phe Dcha	0.01 mmol	HCl 0.04 N in anh. dioxane	1 ml dioxane-HCl 0.04 N (0.04 mmol HCl present)	10 min	100%
2	NPS-Phe Dcha	0.01 mmol	thiourea	0.1 mmol in 0.5 ml DMF + 0.5 ml AcOH	3 min	100%
3	NPS-Phe Dcha	0.01 mmol	thioacetamid	0.1 mmol in 0.5 ml DMF + 0.5 ml AcOH	3 min	100%
4	NPS-Phe Dcha	0.01 mmol	*dl*-thiomalic acid	0.1 mmol in 0.5 ml MeOH	2 h	100%
5	NPS-Phe Dcha	0.01 mmol	*dl*-thiomalic acid	0.1 mmol in 0.5 ml DMF	2 h	100%
6	NPS-Phe Dcha [a]	0.04 mmol	NaSeH	~1 mmol NaSeH in 5 ml AcOH	15 min	NPS-Phe Dcha still present
7	NPS-Phe Dcha	0.02 mmol	selenurea	0.08 mmol in 0.5 ml MeOH/dioxane 1:1	15 min	100%
8	NPS-Phe Dcha	0.01 mmol	*o*-nitrothiophenol	0.01 mmol in 0.5 ml DMF + 0.2 ml AcOH	3 min	100%
9	NPS-Trp Dcha	0.01 mmol	NTBA	0.13 mmol in 0.5 ml dioxane	1 min	100% [b]
10	NPS-Gly Dcha	0.01 mmol	NTBA-OMe	0.05 mmol in 0.7 ml CH_2Cl_2 + traces of Dcha acetate	1 min	100%
11	NPS-Gly-resin	300 mg (0.50 mequiv./g)	NTBA	2 mmol in 2 ml dioxane + 3 mg Dcha acetate	2 h	100% [c] [d]
12	NPS-Gly-resin	300 mg (0.51 mequiv/g)	NTBA-OMe	1 mmol in 2 ml CH_2Cl_2 + 1 mg of Dcha acetate	4 h	100% [d]
13	NPS-Gly-resin	350 mg (0.51 mequiv/g)	*o*-nitrothiophenol	1.3 mmol in 2 ml dioxane + 1 mg Dcha acetate	24 h	81% [d]

[a] NaSeH was partly oxidized by air remaining in the reaction vial; complete exemption of oxygen makes the method cumbersome.
[b] No traces of aryl sulfide after completion of the reaction.
[c] Resin remains yellow; readily washed with aqueous NEt_3 or di-*iso*-propylamine in CH_2Cl_2 or $CH\,Cl_3$ solution.
[d] The free amino acids released were titrated by picric acid, according to [13].

Experimental

General. – The spectrophotometric controls and analyses were performed with *Perkin-Elmer* Model 402 UV.-visible and Model 700 IR. spectrophotometers. – UV. The λ_{max} values are expressed in nm with the corresponding ε values in parenthesis. – IR. (in KBr) are expressed in cm^{-1}. – NMR. were scanned on a *Varian* XL 100–15 Model; chemical shifts are expressed in δ units for $\delta_{TMS} = 0$. (Any deviations will be indicated). M. ps. were determined using a *Fisher-Jones* apparatus and *thermal analyses* recorded on a Differential Thermic Analysis instrument *Mettler* Model TA 2000. Mass spectra were obtained by use of a *Varian* MS 1 spectrometer. Routine *chromatographic analyses* were run on *Merck* Silicagel F 254 thin-layer plates 0.2 mm. With the exception of (Bzl-O)-Thr and -Ser and of (*t*-Bu-O)- Thr and -Ser, all protected amino acids were synthesized.

NPS-amino acids. Amino acids were used in their free, zwitterionic form for the synthesis of all NPS- protected derivatives, according to the procedure of *Zervas et al.* [11]. The reactant *o*-nitrophenylsulfenyl chloride used was in aqueous NaOH soln. Purified N(α)NPS-amino acids were stored as Dcha salts, unless used within 24 h.

Syntheses. – *NPS-GlyO⁻Cs⁺* was prepared from 5 mmol NPS-Gly suspended in 20 ml water, and the pH adjusted gradually to 7.0 with 1 N CsOH. After filtration, the product was evaporated to dryness at 10^{-4} Torr; dissolved in a minimal amount of DMF (~200 ml), crystallization initiated by adding 2 vol. of CH_2Cl_2, and then allowed to proceed overnight at 0°. After washing crystals on the filter with same solvent the yield was 1.31 g (3.65 mmol), 73%. Rf in BuOH/AcOH/H_2O 4:1:1, 0.70, in $CHCl_3$/MeOH 2:1, 0.24. M.p. 177.5°.

NPS-GlyO-resin [12]. 10 g polystyrene resin, *Merrifield*-type, 1% DVB, containing 1 mequiv. of chloromethyl groups per g, were dissolved in 80 ml anh. DMF, 0.8 equiv. of NPS-GlyO⁻Cs⁺ were added and the suspension heated to 50° during 24 h. The substituted resin was washed, on the filter, 3 times with each of the following solvents DMF, EtOH, $CHCl_3$, CH_2Cl_2 and EtOH, in that order, and finally dried at 10^{-4} Torr. A weighed, dry sample, was allowed to react in diluted HCl and the amount of NPS-Cl thus liberated was determined by UV. [398 (3410)], yield 0.55 ± 0.03 mequiv. The amino acid liberated was also determined by difference picrate titration, according to [13]. Diisopropylethylamine picrate was characterized by UV. spectroscopy [354 (14900)].

Trp (2-indole-NPS). To 1.3 mmol Trp (265 mg) 10 ml of anh. 0.1 N HCl in dioxane, 3.9 mmol (705 mg) of *o*-nitrophenylsulfenyl chloride were added in small aliquots, and the mixture allowed to stand overnight at room temp. After pouring 3× vol. of water onto the soln., the precipitate was collected, air dried, dispersed in ether, filtered and dried ultimately at 10^{-4} Torr. Yield (433 mg) 1.2 mmol, 93%; m.p.: 205–206°. Rf in BuOH/AcOH/H_2O 4:1:1. 0.57. Mol. Wt.: 354.39. – UV. (DMSO): 286 (16130), 366 (3608). – IR. (KBr): 3300*s*, 3200–3000*m*, 1620*m*, 1590*m*, 1570*m*, 1510*s*, 1400*m*, 1340*s*. – NMR. (d-DMSO) δ 2.90–3.60, *m*, 4 H; 6.80–8.34, *m*, 9 H; 4.20–4.30, *m*, 1 H; 11.59, *s*, 1 H. – MS.: (Mol. Wt. 413.45): *m/e*: 413 M^+ (23), 354 (7), 283 (100), 253 (11), 236 (23), 200 (23), 154 (16), 138 (23).

Sodium hydroselenide was prepared according to [14] and [15]. To 300 mg (3.8 mmol) of selenium dispersed in 15 ml anh. EtOH and cooled to 0°, 160 mg (4.2 mmol) of sodium borohydride were added progressively, keeping the reaction mixture under constant nitrogen flow. The initially black dispersion turned brownish-red and changed into a clear, almost colorless solution in approx. 15 min; this solution was used as such for the cleavage of N(α)-NPS-amino acids.

4,4′-Dithio-bis(3-nitrobenzoic acid) was prepared according to the method of *Havlik & Kharasch* [10] and the final product crystallized successively in dioxane/petroleum ether and in dioxane/benzene. Rf in $CHCl_3$/MeOH 2:1, 0.11. Mol. Wt.: 396.35. Thermal analysis: exotherm. dec. at 299°.

3-Nitro-4-mercapto-benzoic acid (**1**) 5.8 mmol (2.3 g) of 4,4′-dithio-bis(3-nitrobenzoic) acid was suspended in 100 ml of anh. EtOH. Under permanent flow of nitrogen, 10 ml of 2-mercaptoethanol were added, followed by 1 ml of NEt_3. After standing for 30 min at RT., EtOH was evaporated and 150 ml of concentrated HCl, precooled in ice, were added. The precipitate was filtered off, drained and dried under reduced pressure. Yield 1.9 g (9.6 mmol), 82%. Rf in $CHCl_3$/MeOH 2:1, 0.30. Recrystallized twice from dioxane. Mol. Wt.: 199.19. Thermal analysis: decomposes (exotherm.) at 148°. – IR. 3000–2800*m*, 2500*w*, 1680*s*, 1600*s*, 1510*m*, 1412*m*, 1305*s*, 915*m*. – NMR. and MS.: see NTBA-OMe.

Dimethyl 4,4'-dithio-bis(3-nitrobenzoate). A solution of diazomethane was prepared as follows: To 2.068 g (20 mmol) of nitrosomethylurea, dispersed in 50 ml of ether at 0°, 25 ml of an aqueous soln. of 40% KOH (w/w) were added dropwise, keeping the reactor in ice. After standing for 15 min, the aqueous phase was decanted, then extracted with ether and the organic fractions were pooled and dried in the presence of KOH. This ether soln. was kept at 0° and used within 1 h. To 1.5 g (3.8 mmol) of 4,4'-bis-(3-nitrobenzoic acid) dispersed in 30 ml of ether, 50 ml of the diazomethane solution in ether (16.6 mmol) was added, the suspension cooled to 0° and stirred. After all gas had evolved (about 30 min), solvent and excess diazomethane were evaporated under reduced pressure, to give the diester; yield 1.41 g, 86%. Rf in $CHCl_3$ (on nonactivated gel) 0.30; Rf in $CHCl_3$/MeOH 2:1, 0.86; m.p. 206–207°. – UV.: 255 (36308), 277 sh. (25585), 350 (8917). – IR. (KBr): 3000*w*, 1720*s*, 1600*s*, 1520*s*, 1440*m*, 1390*m*, 1340*s*, 1135*w*. – NMR. ($CDCl_3$): 8.96, *d*, 1 H, $J_{2,6} = 2$ Hz; 8.19, *d* × *d*, 1 H, $J_{6,2} = 2$ Hz, $J_{6,5} = 9$ Hz; 7.90, *d*, 1 H, $J_{5,6} = 9$ Hz; 3.97, *s*, 3 H. – MS. (*m/e*): 424 M^+ (40), 393 (12), 314 (22), 212 (100), 196 (47), 180 (10), 164 (24), 156 (17), 118 (10), 74 (27), 59 (25).

Methyl 3-nitro-4-mercapto-benzoate. 300 mg (0.7 mmol) of dimethyl 4,4'-dithio-bis-(3-nitrobenzoate) were dissolved in 15 ml of CH_2Cl_2, 0.1 ml of NEt_3 and 1 ml of 2-mercaptoethanol added and the mixture allowed to react during 30 min. Addition of 15 ml of ether caused unreacted disulfide to precipitate and after filtration, the soln. was evaporated to dryness. The residue was redissolved into 50 ml of water, and after dropwise addition of 100 µl of glacial AcOH, the yellow precipitate was filtered and washed twice with water, then dried thoroughly under vacuum. Yield 250 mg (1.17 mmol), 83%. M.p. 124°. Rf in $CHCl_3$/MeOH 2:1 0.70. NTBA-OMe gives a positive *Ellman* reaction [16] on TLC., provided the plates are lightly sprayed beforehand with an ethanolic soln. of DTNB. Electronic spectra were strongly pH-dependent. – UV. (EtOH): 257 (15130), 278 (11770), 339 (7925); with 15 µl of AcOH added: 257 (19690), 278 (14890), 350 (3240) (batho-hypo-chromic shift). Large shifts occurred when 15 µl NEt_3 were added to the initial alcoholic soln.: 250 (5040), 278 (4320), 337 (18610) (strongly hyperchromic). – IR. (KBr): 3000*w*, 2500*m*, 1690*s*, 1510*s*, 1430*m*, 1320*m*, 1305*s*, 1150*m*, 980*w*. – NMR. ($CDCl_3$): 8.90, *d*, 1 H, $J_{2,6} = 2$ Hz; 8.07, *d* × *d*, 1 H, $J_{6,2} = 2$ Hz, $J_{6,5} = 9$ Hz; 7.53, *d*, 1 H, $J_{5,6} = 9$ Hz; 3.97, *s*, 3 H. – MS. (Mol. Wt. 213.21): 213 M^+ (22), 196 (25), 182 (11), 118 (34), 32 (34), 28 (100).

1-Nitro-2-mercapto-benzene: To 4 g (13 mmol) of 2-nitrophenyl-disulfide suspended in 75 ml MeOH, 2 ml of 2-mercapto-ethanol and 0.1 ml of NEt_3 were added, under nitrogen flow, and the reaction allowed to proceed for 4 h. The suspension turned brownish-red and slowly cleared to a homogeneous phase. MeOH was evaporated, the remaining soln. diluted with 50 ml of 1 N HCl, precooled in ice, and the pale yellow precipitate was filtered, washed and dried under vacuum. Yield 3.3 g (21.5 mmol), 82%. After several recrystallizations from CCl_4/petroleum ether, m.p. 56°. Rf in $CHCl_3$/MeOH 2:1, 0.82; in $CHCl_3$ (non activated gel), 0.67; in BuOH/AcOH/H_2O 4:1:1, 0.89. Mol. Wt. 189.62.

Methyl 3-nitro-4-chlorosulfenyl-benzoate. To 1.5 g (3.53 mmol) of dimethyl 4,4'-dithio-bis(3-nitro benzoate) (prepared according to [10]) dispersed in 25 ml of CCl_4, a few crystals of iodine were added, and chlorine gas was allowed to bubble through until complete solution had occurred. After standing 4 h at RT., a finely divided precipitate appeared and the mixture was left overnight at 4°. The solvent was then evaporated to dryness and the residue kept at 10^{-4} Torr for 10 h to give a yellow solid. Yield 820 mg (3.31 mmol), 46.7%. Recrystallized from EtOH. M.p. 181°. Mol. Wt.: 247.66. – UV. (EtOH): 262 (16750), 293 (9390). Visible: 392 (3600). – IR. KBr): 3130*w*, 2900*w*, 1730*s*, 1600*s*, 1500*s*, 1430*m*, 1300*s*, 1240*s*, 1140*s*, 750*s*. – NMR. ($CDCl_3$): 8.95, *d*, 1 H, $J_{2,6} = 2$ Hz; 8.40, *d* × *d*, 1 H, $J_{6,2} = 2$ Hz, $J_{6,5} = 9$ Hz; 8.09, *d*, 1 H, $J_{5,6} = 9$ Hz; 4.01, *s*, 3 H. – MS. (*m/e*): 249 (35), 247 (100) M^+, 212 (57), 186 (60), 165 (50), 148 (42), 119 (21), 107 (25), 103 (42), 95 (42), 69 (17), 63 (46).

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88. Untersuchungen über synthetische Arzneimittel 9- und 10-Oxo-Derivate von 9,10-Dihydro-4*H*-benzo[4,5]cyclohepta-[1,2-*b*]thiophenen

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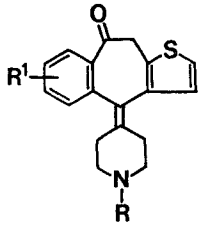
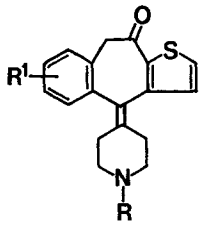
(24. I. 76)

Synthetical pharmaceutics. 9- and 10-Oxo derivatives of 9,10-dihydro-4*H*-benzo-[4,5]-cyclohepta[1,2-*b*]thiophenes. – *Zusammenfassung.* Verschiedene Synthesen zur Herstellung von 9-Oxo-9,10-dihydro-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophenen **17** und 10-Oxo-9,10-dihydro-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophenen **18** (**25**), mit der 1-Alkyl-4-piperidyliden-Gruppe in 4-Stellung, ausgehend von 9,10-Dihydro-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-4-onen **1** bzw. Folgestufen davon, werden beschrieben. Als weitere Derivate sind die N-Oxide **26**, die 9- und 10-Hydroxy-Derivate **27** und **28**, die 9- und 10-Oxime **29** und **30** sowie ein 9,10-Diketon **31** hergestellt worden.

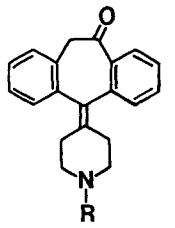
1. Einleitung. – In Weiterführung der Arbeiten auf dem Gebiet des 9,10-Dihydro-4*H*-benzo[4,5]cyclohepta[1,2-*b*]thiophen-Tricyclus [1] wurden die 9- und 10-Oxo-Derivate mit der 1-Alkyl-4-piperidyliden-Gruppe in 4-Stellung hergestellt (**A** und **B**).



A



B



C

In der Literatur [2] sind kürzlich ähnliche Ketone des 5*H*-Dibenzo[*a*,*d*]cyclohepten-Tricyclus (**C**) beschrieben worden. Bei unseren verschiedenen Synthesewegen [3] sind wir von den tricyclischen Ketonen (**D**) ausgegangen, welche in 9- oder 10-