benzene was approximately 0.55 times as reactive as benzene. The relative rates suggest that a highly reactive electrophile is produced during the reaction. Such reactive attack by electrophiles are relatively nonselective for aromatic compounds that are activated or deactivated by substituents, and therefore, relative rates are usually within the same order of magnitude. A competitive rate experiment for acetylation also gave the expected order of reactivity for benzene, toluene, and chlorobenzene. The relative rates were 1, 37, and 0.015, respectively. Here the substituents are exerting more kinetic control in the reaction.

In all of the experiments described so far the $Al_2Cl_7^-$ was present in large excess, because the melt was used as the solvent. True catalysis by the $Al_2Cl_7^-$ was demonstrated by a series of turnover experiments where the composition of the melt was adjusted so that it provided less $Al_2Cl_7^-$ than the amount of substrate. A reaction that contained benzene, acetyl chloride, and $Al_2Cl_7^-$ in the proportions of 1.1:1.0:0.5, respectively, afforded complete conversion of the benzene to acetophenone in less than 5 min. A second reaction using the proportions 1.1:1.0:0.1 still afforded a yield of acetophenone greater than the $Al_2Cl_7^-$ would permit if consumed, but at a rate 20 times slower than the first experiment.

Conclusion

The imidazolium chloroaluminate room-temperature molten salts provide a medium for Friedel–Crafts alkylations and acylations that is both the solvent and catalyst. Because the Lewis acid species in the melt is well characterized, the catalyst for the Friedel–Crafts reactions can be clearly identified as $Al_2Cl_7^-$. This is consistent with the fact that pure $AlCl_3$ is not an effective catalyst in Friedel–Crafts reactions, why traces of a co-catalyst are necessary, and why a twofold excess of $AlCl_3$ promotes greater yields.[23] In the case of acetylation, the reactive electrophile can be isolated as a crystalline material. We propose the following mechanism for the acylation of an aromatic compound, Ar–H, by the acyl chloride, RCO–Cl, catalyzed by the melt:

$$RCO\text{–}Cl + Al_2Cl_7^- \rightarrow RCO^+ + 2AlCl_4^-$$

$$RCO^+ + Ar\text{–}H \rightleftharpoons H\text{–}Ar^+\text{–}CO\text{–}R$$

$$H\text{–}Ar^+\text{–}COR + AlCl_4^- \rightleftharpoons Ar\text{–}CO\text{–}R + HCl + AlCl_3$$

$$AlCl_3 + AlCl_4^- \rightarrow Al_2Cl_7^-$$

$$RCO\text{–}Cl + Ar\text{–}H \rightarrow Ar\text{–}CO\text{–}R + HCl$$

We believe that the first and last steps are very rapid and virtually quantitative. The cationic intermediate shown as a product of the first step may not be the completely ionized acylium, but instead the reaction may produce other species of equivalent stoichiometry and reactivity.

Experimental Section

The 1-methyl-3-ethylimidazolium chloroaluminate melts were prepared as described earlier.[4] All melt preparations were done in a helium- or argon-filled glovebox maintained at <10 ppm combined water and oxygen. The organic reactions were performed on the bench top under a stream of dry nitrogen.

General Friedel–Crafts reaction procedure: The aromatic Friedel–Crafts substrate was added to a weighted quantity of melt (0.1–5 g). The alkylating agent was added either at room temperature or at the reflux temperature of the alkyl chloride. For kinetics determinations an aliquot of the reaction mixture (usually 0.1 or 0.2 mL) was removed, added to 2 mL of water to quench the reaction, and made basic with 6 m NaOH. The mixture was extracted with 5 mL of diethyl ether and dried over anhydrous $MgSO_4$.

The products were analyzed by GC/MS on a Hewlet–Packard Model 5985 mass spectrometer equipped with a 3 ft column packed with 2% OV-101. NMR spectra were obtained on a Varian T-60 or JEOL FX90Q spectrometer.

Registry No. Benzene, 71-43-2; methyl chloride, 74-87-3; ethyl chloride, 75-00-3; *n*-propyl chloride, 540-54-5; *n*-butyl chloride, 109-69-3; cyclohexyl chloride, 542-18-7; benzyl chloride, 100-44-7; 1-methyl-3-ethylimidazolium chloroaluminate, 87587-77-7; acetyl chloride, 75-36-5.

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Monitoring and Optimization of Deprotection Reactions of Peptides by Direct Sampling with Fast Atom Bombardment Mass Spectrometry†

Klaus Eckart and Helmut Schwarz*

Institut für Organische Chemie, Technische Universität Berlin, D-1000 Berlin 12, FRG

Günther Becker and Horst Kessler*

Institut für Organische Chemie, Johann-Wolfgang Goethe Universität, D-6000 Frankfurt/Main 50, FRG

Received June 28, 1985

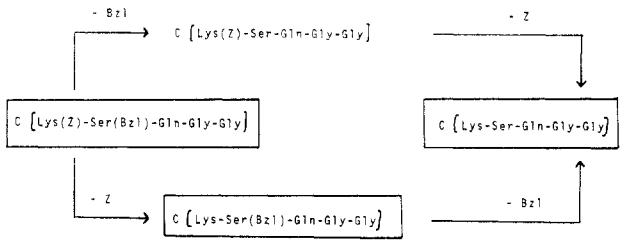
Deprotection is often one of the most critical reactions in peptide chemistry. Direct monitoring of these reactions using fast atom bombardment (FAB) mass spectrometry proved a suitable method to optimize reaction conditions. The use of the micromolar reaction scale efficiently reduces the amount of fully protected peptide needed. All investigations were carried out with cyclic analogues of biologically active peptides.

In organic chemistry and particularly in peptide chemistry the use of protecting groups is essential for the success of a synthesis, but their removal often presents a serious challenge. General procedures invariably have to be specifically adapted and optimized for each individual case to prevent incomplete deprotection and various side reactions. The optimization is frequently extremely time consuming and usually severely limited by the amounts needed to detect, separate, and characterize the different

† Dedicated to Professor Ferdinand Bohlmann, TU Berlin, on the occasion of his 65th birthday.

0022-3263/86/1951-0483$01.50/0

Scheme I. Possible and Observed (in Boxes) Reaction Components for the Hydrogenolysis of Peptide 1



reaction products obtained in each trial run.

FAB mass spectrometry[1] is an ideal analytical tool for monitoring reactions of this kind because the reaction products can be distinguished by their difference in molecular weight. Furthermore, the sample to be analyzed is taken directly from the reaction mixture without any prior separation and/or other intervening processes. [While it is known[2] that a quantitative mixture analysis of molecules of moderately to highly concentrated solutions (up to 10%) by monitoring the MH^+ ion intensities with FAB is in general not possible, this does not apply for diluted solutions (≤1%). For example, we have observed that mixtures of protected (**2**) and the corresponding unprotected peptide over a range of 2:1–10:1 molar ratios were excellently reproduced by the intensities of the corresponding MH^+ ions in the FAB mass spectra.]

Direct sampling becomes possible because in FAB mass spectrometry involatile compounds such as peptides are generally mixed with glycerol or related viscous solvents[3] and then directly subjected to ionization. Deprotection reactions were applied to various cyclic analogues of biologically active peptides bearing examples of all benzyl-type protecting groups [(benzyloxy)carbonyl (=Z), benzyl esters (=OBzl) and benzyl ethers (=Bzl)]. These protecting groups are often favored because of their stability during synthesis and the wide range of deprotection conditions that have been employed successfully. Their removal was carried out with proteolytic and hydrogenolytic systems on the micromolar scale in close parallel to full-scale procedures.

Results

In Scheme I the different intermediate species occurring during the deprotection of a compound bearing more than one protecting group are shown for compound **1** (cf. Table I). Especially in cases of more than two protecting groups, a careful consideration of all possible reaction pathways is helpful (even though in the example chosen, the reaction pathway can clearly be predicted from known reactivities). The different chemical intermediates can be recognized through their molecular weight. Their relative intensities can be monitored through sampling the reaction every few minutes.

A plot of the relative signal intensities of $[M + H]^+$ as a function of the reaction time (Figure 1) gives a clear idea of the reactions taking place. Equally, side reactions such as the acetylation of Ser and Thr (cf. Table I, reactions 1, 8, 13, and 17) can be monitored. Another common side

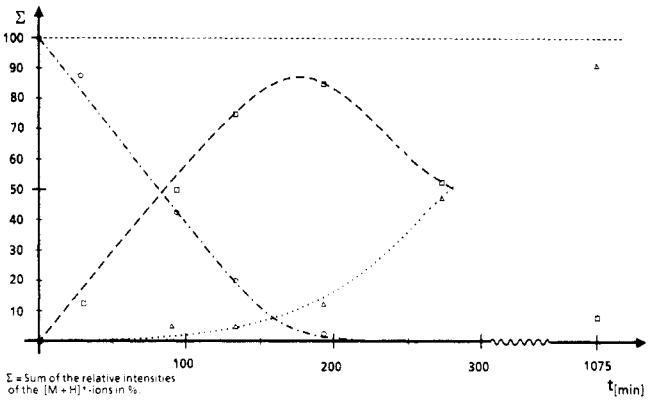


Figure 1. Time dependence of the removal of the protection groups of peptide 1: (○···) c[Lys(Z)-Ser(Bzl)-Gln-Gly-Gly]; (□---) c[Lys-Ser(Bzl)-Gln-Gly-Gly]; (△···) c[Lys-Ser-Gln-Gly-Gly].

reaction, the N → O acyl shift of the Ser hydroxyl group in proteolytic reaction systems,[5] leads to depsipeptides with characteristic fragmentation pattern in the FAB mass spectra.[6]

Great care must be taken to distinguish between fragments resulting from chemical reactions and those generated by fragmentation processes occurring within the mass spectrometer. This differentiation can be accomplished through careful study of the fragmentation patterns of the protected peptides in nonreactive solvent systems. The ratio of fragments generated within the mass spectrometer remains constant throughout the deprotection reaction, while the ratio of the signals corresponding to the different chemical compounds involved changes (cf. Figure 1). If these criteria are kept in mind, highly reproducible and reliable results can be obtained.

Compounds **1–5** are cyclic analogues of the thymic hormone "thymic serum factor"[7] and show very similar chemical reactivity. Of the three proteolytic systems tested (cf. Table I), only the HBr/TFA procedure[8,9] gave good deprotection without side products, but the timing of the deprotection reaction proved to be critical: Undue extension of the reaction time leads to side products. Catalytic hydrogenation is more suitable, though reaction times are fairly long. Reaction times vary with ring size and seem less dependent on the reaction scale (cf. Table I, reactions 5 and 6).

The hydrogenolysis of the biologically active thymopoietin[10] analogue **7**[11] proceeded cleanly and reactions 20 and 21 show the influence of the ratio of catalyst to substrate on the reaction time as it was increased from 5 min to 2 h for complete deprotection.

Larger scale deprotection of the cyclic enkephaline[12] analogue **6**[13] led to 30% of acetylated side product with the HBr/HOAc procedure. The results could be reproduced on the micromolar scale, and reaction conditions were developed that avoid this side reaction (cf. Table I, reactions 17–19).

In this manner, mass spectrometry cannot only be used for a general test of deprotection conditions for a given

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Table I. List of the Deprotection Reactions Studied

peptide	reaction number	am, mg/μmol	reacn conditions[i]	T_R,[h] min	results
c[Lys(Z)–Ser(Bzl)–Gln–Gly–Gly] (1)	1	0.7/1.0	10 equiv (2 μL) of HBr/HOAc; *a* RT	120	incomplete removal of Z and Bzl; generation of Ser–acetate
	2	0.7/1.0	10 equiv (2 μL) of HBr/HOA,[a] 0 °C	120	incomplete removal of Z
	3	0.7/1.0	20 equiv of MSA;[b] 22% TFA; RT	40	gives mainly the MSA adduct of the deprotected compounds; desanylation with NH_3 led to fragmentation of the peptide
	4	0.7/1.0	HBr/TFA;[c] 0 °C	45	complete deprotection; extension of reaction time leads to side products
	5	0.8/1.2	150% Pd/C,[d] 60 μL of solvent[e]	720	complete removal of Z within 60 min; slow hydrogenolysis of Bz ethers free from side products
	6	6.8/10	100% Pd/C,[d] 600 μL of solvent[e]	1400	complete removal of Z in 240 min; of Bzl as in reaction 5
c[Lys(Z)–Ser(Bzl)–Gln–(D)Ala–(D)Ala] (2)	7	1.2/1.7	100% Pd/C;[d] 60 μL of solvent[e]	720	as in reaction 5
c[Lys(Z)–Ser(Bzl)–Gln–Gly–Gly–Ser(Bzl)] (3)	8	0.9/1.1	10 equiv (3 μL) of HBr/HOAc;[a] RT	120	removal of Z within 60 min; only partial removal of either one of the Bzl groups, followed by acetylation of Ser(OH)
	9	0.9/1.1	10 equiv (3 μL) of HBr/HOAc;[a] 0 °C	120	incomplete removal of Z
	10	0.9/1.1	20 equiv MSA;[b] 25% TFA; RT	40	gives mainly the di–MSA adduct of the deprotected compound
	11	0.9/1.1	100% Pd/C;[d] 30 μL of solvent[e]	150	complete removal of all protecting groups without side reactions
c[Lys(Z)–Ser(Bzl)–Gln–Gly–Gly–Thr] (4)	12	1.8/2.1	100% Pd/C;[d] 60 μL of solvent[e]	200	as in reaction 11
c[Ala–Lys(Z)–Ser(Bzl)–Gln–Gly–Gly–Ser(Bzl)] (5)	13	1.0/1.1	10 equiv (3 μL) of HBr/HOAc;[a] RT	120	as in reaction 8
	14	1.0/1.1	10 equiv (3 μL) of HBr/HOAc;[a] 0 °C	120	as in reaction 9
	15	1.0/1.1	20 equiv MSA;[b] 25% TFA; RT	30	as in reaction 10
	16	1.0/1.1	100% Pd/C;[d] 30 μL of solvent[e]	120	as in reaction 11
	18	0.2/0.3	HBr/HOAc;[g] 1 μL; RT	180	complete removal, only small traces of the side products found in reaction 17
	19	0.5/0.6	100% Pd/C;[d] 20 μL of solvent[e]	540	almost complete deprotection within 30 min; no acetylation, after prolonged reaction small traces of the dehydro–alanine–cyclopentapeptide and further of the alanine–cyclopeptide from the side reaction described in reaction 17
c[Arg(NO_2)–Lys(Z)–Asp(OBzl)–(D)Val–Tyr] (7)	20	0.3/0.3	160% Pd/C;[d] 30 μL of solvent[e]	5	see comment for reaction 11
	21	0.7/0.8	10% Pd/C;[d] 30 μL of solvent[e]	120	see comment for reaction 11

[a] 1 mol of peptide is dissolved or suspended in 2 μL of glacial acetic acid, and 10 mol equiv of 33% HBr in glacial acetic acid per protecting group is added. [b] 1 μmol of peptide is dissolved in TFA (trifluoroacetic acid), and 20 mol equiv (1.3 μL) of methanesulfonic acid (=MSA), containing 2 vol % anisole, is added. The amount of TFA is calculated to give the concentration of MSA in TFA given in the table. [c] The compound is dissolved in 100–150 μL of TFA, and HBr is led through the solution by using a thin-glass capillary to effect a steady gas flow. TFA is added every few minutes to compensate for evaporation. [d] The catalyst used was 10% Pd/C. The ratio catalyst/substrate is given in wt % peptide. [e] As solvent a 1:1 solution of glacial acetic acid in water was used. [f] 33% HBr in glacial acetic acid was added in large excess without prior administration of solvent. [g] 0.5 μL (10 equiv) of 33% HBr in glacial acetic acid was added to the sample after dissolving it in 1 μL of glacial acetic acid. This is twice the amount of solvent used as compared to reactions 1, 2, 8, 9, 13, and 14 (cf. *a*). [h] t_R = reaction time in minutes. [i] RT = room temperature.

class of compounds, as shown for compounds 1, 3, and 5, but also to study and optimize specific reactions either with hydrogenolytic systems as shown with compound 7 or with proteolytic systems as shown with the enkephaline analogue 6.

It should be mentioned, that the products of all successfully optimized deprotection reactions mentioned above were in fact isolated. The amounts (0.5 mg) suffice to give immediate access to further analytical data such as biochemical activities or chiral amino acid analysis. The latter is of great interest, as mass spectrometry, in general, supplies no information on enantiomeric purity.

Discussion

The monitoring of chemical reactions depends on the separation and identification of the different reaction products and intermediates. Usual methods for monitoring are TLC and HPLC. Both depend on the previous development of separation conditions. The identification of the different reaction products is usually achieved by a separate workup and isolation procedure. This involves automatically loss of time and material, while the amount needed finally depends on the method of identification.

By the application of FAB mass spectrometry, reactions can be monitored in real-time and components be identified immediately, and the sensitivity lies in the nanomolar range.

Consequently, we feel that the direct application of FAB mass spectrometry allows deprotection reactions to be optimized much more easily and efficiently than conventional methods.

Deprotection reactions of synthetic peptides can, of course, also be monitored via TLC or HPLC. But appropriate solvent systems have to be found that ensure complete separation of all the different reaction products and intermediates, which is usually followed by a separate identification step. We feel, that these alternative procedures are comparatively more time consuming than the direct application of FAB-MS.

Experimental Section

The purity of all protected peptides was assessed through amino acid analysis, TLC, HPLC, FAB-MS, and NMR spectroscopy. All reactions were carried out in Eppendorf micro test tubes 3815. The reaction mixtures were stirred occasionally and before sampling by using a mechanical shaker (Heidolph, Type Reax 1DR).

Hydrogenolysis. Hydrogen was led through a thin-glass capillary into the reaction mixture. To obtain constant gas flow, the hydrogen pressure was controlled with a microvalve. For workup, the reaction mixture was first diluted to 0.5 mL and then the catalyst concentrated by centrifugation for 15 min at 3500 rpm. The clear solution was pipetted and the catalyst suspended again in 0.5 mL of solvent. The process was repeated, and the combined solutions were centrifuged again to remove traces of catalyst. The solution was then lyophylized.

HBr/TFA Procedure. The reaction was carried out as described in Table I. The gas flow was regulated manually. Though cooling was supplied, the rapid evaporation of TFA made constant additions of new solvent necessary: as a rule 100 μL were added every 5 min with an Eppendorf pipet.

Other proteolytic reactions were carried out under argon atmosphere as described in Table I.

Sampling. All samples were drawn directly from the reaction mixture by quick immersion of a thin-glass capillary. The sample, mixed with equal amounts of glycerol, was then centered on the tip of the FAB probe of the mass spectrometer.

FAB mass spectra were recorded with a Vacuum Generator (VG) ZAB-3HF mass spectrometer (BEB configuration) equipped with a VG 250/11 data system at the following conditions: ion source pressure, 5×10^{-6} torr; ion source temperature, 20 °C; xenon as FAB gas, 8 keV xenon atoms; 0.1 mA emission current in the FAB gun; 8kV acceleration voltage for the secondary ions; 1300 mass resolution in the double focusing mode; scan speed, 5 s/decade.

Acknowledgment. Financial support of the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie is gratefully acknowledged.

Registry No. 1, 99922-03-9; 2, 99901-80-1; 3, 99901-81-2; 4, 99901-82-3; 5, 99901-83-4; 6, 99901-84-5; 7, 96393-61-2.

Application of a Transition-Metal-Mediated Stereospecific Michael Reaction Equivalent to the Synthesis of Alloyohimbone

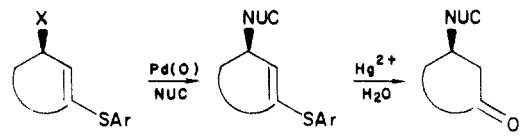
Stephen A. Godleski*[1] and Edwin B. Villhauer

Department of Chemistry, University of Rochester, Rochester, New York 14627

Received August 12, 1985

A stereospecific Michael reaction equivalent, based on a sulfur-substituted (π-allyl)palladium intermediate, has been applied as the key step in a synthesis of alloyohimbone (2). The use of the identical π-allyl precursor in the absence of palladium provided an entry into the opposite stereochemical series and could potentially be used in a synthesis of yohimbone (3).

We had previously reported a palladium-mediated equivalent to the Michael reaction which allowed complete stereospecificity by virtue of the intermediacy of a (π-allyl)Pd complex.[2,3]



This feature was particularly significant because of the notoriously poor stereochemical control associated with the "native" Michael reaction.[4] In tandem with a Diels–Alder reaction this process was also shown[3] to provide the alternative stereochemical outcome to that typically pre-

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