Reactions of \( \beta \)-Propiolactone with Nucleobase Analogues, Nucleosides, and Peptides

**IMPLICATIONS FOR THE INACTIVATION OF VIRUSES**

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Modern vaccines are based on sound scientific knowledge and rational design of all the development stages. Nevertheless, chemical inactivation of viral vaccines is still a matter of trial and error without much detailed knowledge of the chemistry involved. \( \beta \)-Propiolactone and formaldehyde are compounds that are frequently used for the inactivation of viruses and the production of vaccines, such as influenza, polio, and rabies vaccines (1–12). A considerable problem in the production of vaccines is the loss of immunogenicity during the inactivation process (13–15). To prevent destruction of important epitopes and receptor sites in viruses, chemicals with a different specific reactivity have been utilized for inactivation (8, 10, 11, 13, 16–19). According to the literature, formaldehyde reacts primarily with proteins (19, 20), whereas \( \beta \)-propiolactone modifies mainly DNA or RNA (21–23). Therefore, it is expected that \( \beta \)-propiolactone will maintain a high immunogenicity during the inactivation of viruses.

\( \beta \)-Propiolactone is a hazardous compound that may cause cancer (24–30). It consists of an almost planar highly strained four-membered ring (Fig. 1). Due to this strain, \( \beta \)-propiolactone reacts readily with nucleophiles. Extensive studies have been performed in the past to determine the reactivity with many nucleophiles, e.g. water, inorganic salts, amines, alcohols, thiols, and carboxylic acids (31–38). The reaction of \( \beta \)-propiolactone is a typical example of an \( S_2 \) reaction in which the rate of disappearance depends on the concentrations of a particular nucleophile and \( \beta \)-propiolactone according to Equation 1.

\[
d[BPL]/dt = k[BPL][nucleophile] \tag{Eq. 1}
\]

In aqueous media, water is an important nucleophile for \( \beta \)-propiolactone, which has at room temperature a half-life of about 3–4 h (39). The half-life can be decreased in the presence of other nucleophiles. Both the concentration and nature of the nucleophile are important for the conversion rate (40). When a mixture of nucleophiles is used, the rate of disappearance of \( \beta \)-propiolactone equals the summation (41, 42),

\[
d[BPL]/dt = k_1[BPL][nucleophile 1] + k_2[BPL][nucleophile 2] + k_3[BPL][nucleophile 3] + \cdots \tag{Eq. 2}
\]

The situation becomes more complicated in the case of multiple nucleophilic sites present in one molecule, e.g. in a DNA or protein molecule (43, 44). The reactivity of a particular nucleo-
philic group in such molecules is influenced by global parameters, e.g. pH and temperature, and by local circumstances, e.g. the accessibility and non-covalent interactions (45, 46).

β-Propiolactone can react with nucleophiles in two ways, resulting in alkylated and acylated products (Fig. 1). According to the Hard Soft Acids Bases theory, hard nucleophiles, such as primary amino groups (RNH₂), will react with the hard acyl carbon, whereas soft nucleophiles, such as thiol groups (RSH), will give alkylated products (47). Hardness or softness of a nucleophile is also influenced by local parameters. For example, hydrogen bonding can increase softness of primary amino or thiol groups, which may result in increased alkylation. Also, pH plays a role; when a thiol group becomes deprotonated, its softness decreases. The same holds for water, which becomes a harder nucleophile upon deprotonation. Generally, the microenvironment of the nucleophile influences the hardness and, by that, the reactivity of the nucleophilic group (48).

Not all reactions with β-propiolactone will lead to inactivation of a virus. The main reaction during inactivation will be the reaction with water to give a non-toxic compound: 3-hydroxypropionic acid. Also, salts and buffer components have a large effect on the conversion of β-propiolactone (34). Virus inactivation will only occur after reaction of β-propiolactone with viral constituents e.g. DNA/RNA and protein (23, 38, 49–53). Although a number of chemical modifications induced by β-propiolactone have been elucidated, the nature of all possible reactions is still largely unknown. Current knowledge is not sufficient to develop an accurate inactivation procedure with β-propiolactone to produce a viral vaccine that preserves the highest immunogenicity.

The purpose of the present work was to elucidate by using model compounds the reactions that occur during inactivation of viruses with β-propiolactone. The reactivity of β-propiolactone with (i) plain buffers, (ii) with nucleobase analogues and nucleosides, and (iii) with amino acid residues were investigated in detail. The reaction conditions used in this study were based on inactivation processes applied for viral vaccines, such as influenza and rabies vaccines (10, 54, 55). Frequently used buffers during inactivation of viruses are phosphate, citrate, and HEPES. The kinetics of hydrolysis of β-propiolactone was examined in these buffers. Also products formed during the reaction with buffer components were identified by nuclear magnetic resonance spectroscopy (NMR). Second, a systematic study was performed to determine the reactivity of particular reactive sites in nucleotides. A set of nucleobase analogues and nucleosides was used to identify and quantify by NMR the different chemical modifications that occur during β-propiolactone treatment. Furthermore, the reaction of β-propiolactone was investigated with various amino acid derivatives and synthetic peptides. The peptides synthesized had an amino acid sequence Ac-VTLXVTR-NH₂ in which one amino acid residue (X) varies. The reaction of amino acid derivatives was determined by NMR, whereas the conversion of peptides was monitored by tandem reversed-phase liquid chromatography mass spectrometry (LC/MS). In this paper we give an overview of the major conversion products that can be formed during inactivation of viruses by β-propiolactone.

**Experimental Procedures**

**Chemicals**—Nucleic acid analogues 2-aminopyridine, 2-aminopyrimidine, aniline, 7-azaindole, benzimidazole, cytidine, deoxyguanosine, deoxyadenosine, hypoxanthine, imidazole, indole, thymidine, pyridine, pyrimidin-4(3H)-one, 2-pyrrole, and pyrrole were obtained from Sigma. Amino acid analogues N-acetylarginine methyl ester (Ac-Arg-OMe), N-acetylaspartic acid methyl ester (Ac-Asp-OMe), N-acetylcysteine methyl ester ((Ac-Cys-OMe)₃), N-acetylglutamic acid methyl ester (Ac-Glu-OMe), N-acetylhistidinyl methlamide (Ac-His-NHMe), N-acetyllysylsine methyl ester (Ac-Lys-OMe), N-acetylmethionine methyl ester (Ac-Met-OMe), N-acetyltirosine amide (Ac-Tyr-NH₂), and N-acetyltryptophan (Ac-TrpOH) were purchased from Bachem Ag (Bubendorf, Switzerland). N-Acetylcysteine methyl ester (Ac-Cys-OMe) was from Sigma NMR. HEPES, sodium dihydrogen phosphate (NaH₂PO₄·2H₂O), disodium hydrogen phosphate (Na₂HPO₄), trisodium citrate dihydrate, citric acid, and 3-isopropionic acid were from Sigma. β-Propiolactone was obtained from Acros (Geel, Belgium). β-Propiolactone is reasonably anticipated to be a human carcinogen (56). Handling of this compound should be performed using an efficient hood while wearing adequate protection.

**Reactions of β-Propiolactone**

**Reactions with Buffers**—Three citrate buffers with a pH of 6.6, 7.2, or 7.8 were prepared by mixing 0.125 M sodium citrate with 0.125 M citric acid, three HEPES buffers with a pH of 6.6, 7.2, or 7.8 by adjusting 0.1 M HEPES solution with 1 M sodium hydroxide, and three phosphate buffers with a pH of 6.6, 7.2, or 7.8 combining 0.1M sodium citrate hydroxide, and three phosphate buffers with a pH of 6.6, 7.2, or 7.8 combining 0.1 M Na₂HPO₄ with 0.1 M NaH₂PO₄. A phosphate-buffered saline (PBS) solution (155 mM NaCl, 2.71 mM Na₂HPO₄, and 1.54 mM KH₂PO₄, pH 7.2) was obtained from Invitrogen. The reaction was started by adding 1 μl of β-propiolactone to 990 μl of a buffer. The resulting mixture was briefly vortexed, and 500 μl was diluted with 200 μl of the internal standard trimethylsilyl d₄ propionic acid sodium salt (0.075% in D₂O). ¹H NMR spectra were recorded at 735-s intervals during 6–15 h. Relevant signals were integrated. From the integrals, the half-life of β-propiolactone was calculated using an exponential decay model (based upon Equation 3).

\[
d_{[BPL]/dt} = k_1[BPL][H₂O] + k_2[BPL][buffer]. \quad \text{(Eq. 3)}
\]

**Reactions of β-Propiolactone with Nucleobase Analogues and Nucleosides**—The nucleic acid analogues were dissolved in D₂O to final concentrations of 20 mM. A reaction mixture was prepared by successively adding 1500 μl of a nucleobase analog solution, 460 μl of D₂O, and 40 μl of 1.0 M potassium phosphate, pH 9.3. The pH of the samples was adjusted to 9.3 using sodium hydroxide of 1.0 M (the high pH was chosen to increase the amount of reaction products). The reaction was started by

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**FIGURE 1.** Two possible reaction paths of a nucleophile (Nu) with β-propiolactone leading to (I) acylated and (II) alkylated products.
adding 2 µl of β-propiolactone. After mixing, the solution was incubated for 16 h at 22 °C. Samples were stored at 4 °C before analysis by NMR.

**Reactions of β-Propiolactone with Amino Acid Derivatives**—The amino acid analogues were dissolved in D$_2$O to final concentrations of 20 mM. A reaction mixture was prepared by successively adding 1500 µl of an amino acid solution, 460 µl of D$_2$O, 40 µl of 1.0 M potassium phosphate, pH 9.0, and 2 µl of β-propiolactone. After the addition of potassium phosphate and β-propiolactone, the solution was homogenized by gentle mixing. A sample of 500 µl was immediately taken and mixed 200 µl of trimethylsilyl d$_4$ propionic acid. $^1$H NMR spectra were recorded at 735-s intervals during 6–15 h.

**Peptides**—Peptides were synthesized as described previously (19). In brief, the peptides were produced on a 30-mmol scale by using an automated multiple peptide synthesizer equipped with a 96-column reaction block (SYRO II, Fa. MultiSynTech, Gmbh, Witten, Germany). Couplings were performed with Fmoc$^2$-amino acid (90 mmol), benzotriazolyloxy-tris-[N- pyrrolidino]phosphonium hexafluorophosphate (90 mmol), and N-methylmorpholine (180 mmol). The Fmoc group was affected with trifluoroacetic acid (TFA)/water (19/1, v/v), except for cysteine-, methionine- and tryptophan-containing peptides, which were treated with TFA/ethane thiol (19/1, v/v).

The peptides were purified by reversed-phase (C8 column) high performance liquid chromatography, and their identity was confirmed by LC/MS. Before use, peptides were dissolved in D$_2$O to final concentrations of 20 mM. A reaction mixture was prepared by successively adding 100 µl of an amino acid solution, 460 µl of water. The reaction was started by mixing. A sample of 500 µl was immediately taken and mixed 200 µl of trimethylsilyl d$_4$ propionic acid. $^1$H NMR spectra were recorded at 735-s intervals during 6–15 h.

**Reactions of β-Propiolactone with Peptides**—For the reaction of peptides with β-propiolactone, 10 µl of a 1 mM peptide solution and 20 µl of 0.1 M potassium phosphate of pH 3.0, 5.0, 7.0, or 9.0 were added to 70 µl of water. The reaction was started by adding 5 µl of 320 mM β-propiolactone in water (freshly prepared). After mixing, the solution was incubated for 16 h at 4 °C and then 2 h at 37 °C. Samples were stored at −20 °C before analysis by LC/MS.

**Reactions of Iodopropionic Acid with Peptides**—For the reaction of peptides with 3-iodopropionic acid, 10 µl of a 1 mM peptide solution and 20 µl of 0.1 M potassium phosphate of pH 9.0 were added to 60 µl of water. The reaction was started by adding 10 µl of 400 mM 3-iodopropionic acid in water. After mixing, the solution was incubated for 14 days at 35 °C. Samples were stored at −20 °C before analysis by LC/MS.

**Hydrolysis of β-Propiolactone-modified Residues**—After the reaction of peptides with β-propiolactone, 90 µl of 10 mM sodium hydroxide solution was added to 10 µl of the reaction mixture. After mixing, the solution was incubated for 1 h at 37 °C to hydrolyze the base-sensitive modifications. Samples were diluted in 5% (v/v) DMSO and 5% (v/v) formic acid to stop the hydrolysis. Before analysis by LC/MS, samples were stored at −20 °C.

**NMR Measurement**—The samples were analyzed using an NMR spectrometer (JEOL JNM ECP400 spectrometer) operat-

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2 The abbreviations used are: Fmoc, fluorenyl methoxycarbonyl; BPL, β-propiolactone; ΔM, mass increments.
TABLE 1

| Buffer   | pH   | k_1 (H_2O) | k_2 (buffer) | k_{Total}^a | t_{1/2} |
|----------|------|------------|--------------|--------------|---------|
| Citrate  | 6.6  | 5.60E-05   | 4.89E-02     | 0.125        | 9.19E-03 |
| Citrate  | 7.2  | 5.60E-05   | 5.11E-02     | 0.125        | 9.46E-03 |
| Citrate  | 7.8  | 5.60E-05   | 5.20E-02     | 0.125        | 9.58E-03 |
| HEPES    | 6.6  | 5.60E-05   | 2.05E-04     | 0.1          | 3.10E-03 |
| HEPES    | 7.2  | 5.60E-05   | 4.54E-03     | 0.1          | 3.53E-03 |
| HEPES    | 7.8  | 5.60E-05   | 1.96E-03     | 0.1          | 3.38E-03 |
| Phosphate| 6.0  | 5.60E-05   | 9.87E-03     | 0.1          | 4.07E-03 |
| Phosphate| 7.2  | 5.60E-05   | 1.84E-02     | 0.1          | 4.92E-03 |
| Phosphate| 7.8  | 5.60E-05   | 2.38E-02     | 0.1          | 5.46E-03 |
| PBS      | 7.2  | 5.60E-05   | 4.7E-03      | 0.15         | 3.80E-03 |
| Water    | 7.8  | 5.60E-05   | 3.08E-03     | 0.1          | 3.08E-03 |

^aK_{Total} = k_1[H_2O] + k_2[buffer] + k_3[buffer] + [H_2O] = 55 M.

RESULTS AND DISCUSSION

Half-life of β-Propiolactone in Buffer Solutions

The inactivation of viruses is normally performed with β-propiolactone concentrations between 0.025 and 1% (4–160 mM). In this experiment we determined the half-life of 16 mM β-propiolactone in water or buffers containing citrate, phosphate, PBS, and HEPES (Table 1). The half-life of β-propiolactone in water was 225 min at 25 °C, which is in good agreement with the value of 3.5 h reported previously (39). An example of NMR spectra is given in Fig. 2, showing the conversion of 16 mM β-propiolactone in a citrate buffer (pH 7.8, 125 mM). β-Propiolactone (NMR peaks at 4.3 and 3.6 ppm) forms 3-hydroxypropionic acid and imidazole. This phenomenon was observed by NMR when treating an aqueous solution of β-propiolactone with water (Table 1), and no reaction products were detected by NMR at tested pH values (Table 1). In contrast, β-propiolactone was not detected after treatment of indole with β-propiolactone because the electron pair is not involved in the aromaticity of pyridine. Sixty-five percent of the pyridine molecules was converted into N-(2-carboxyethyl) pyridine (NMR: δ 8.88, d, 2H, 8.56, dt, 1H, 8.08, t, 2H, 4.80, t, 2H, 2.92, t, 2H). The proposed structure is given in Table 2. In the case of indole, like pyrrole, no reaction products were observed after treatment of indole with β-propiolactone because the single electron pair of indole is needed for aromaticity (Table 2). As expected, the pyridine-like nitrogen atom N-7 in 7-aza-indole did react with β-propiolactone, whereas the pyrrole-like nitrogen atom N-1 was not modified (Table 2).

Imidazole

In contrast to pyrrole, imidazole was alkylated by β-propiolactone, giving mono- and bis-alkylated products (Table 2). This result is reported previously in the literature (59). Both nitrogen atoms of imidazole, N-1 and N-3, were involved in the alklylation reaction. As a result of tautomerization, the nitrogen N-1 is deprotonated after alklylation at the nitrogen N-3. The electron pair at N-1, which becomes available in the monoalkylated imidazole, reacted with β-propiolactone to form the bis-alkylated product. Moreover, acylation of imidazole occurred, which resulted in 3-hydroxypropionyil imidazole. However, the acylated compound became hydrolyzed in water providing 3-hydroxypropionic acid and imidazole. This phenomenon is the basis of the catalytic effect of imidazole, increasing the rate of hydrolysis of β-propiolactone (Fig. 4). When the reaction of imidazole with β-propiolactone (66 mM imidazole and 36 mM β-propiolactone in 20 mM phosphate buffer, pH 7.8) was recorded by NMR every 5 min, the transient appearance of N-(3-hydroxypropionyl) imidazole could be observed (NMR: δ 3.28, t, 2H, 4.05, t, 2H, 7.12, bs, 1H, 7.7, bs, 1H, 8.42, bs, 1H) together with the mono-alkylated (NMR: δ 7.8, t, 1H, 4.33, t, 2H, 7.32, s, 1H, 7.42, s, 1H, 8.42, s, 1H) and bisalkylated (NMR: δ 7.27, t, 2H, 4.42, t, 2H, 7.48, d, 2H, 8.76, t, 1H, 10H) products. From the NMR spectra, the concentrations of β-propiolactone and its reaction products with imidazole and water were obtained (Fig. 4). Using a model depicted in Fig. 4, reaction rate constants (k_{1-5}) were calculated with k_1 = 5.6-10^{-3}, k_2 = 6.73-10^{-2}, k_3 = 9.89-10^{-3}, k_4 = 6.8-10^{-2}, and k_5 = 6.41-10^{-2}.

Reactions of β-Propiolactone

In heterocyclic compounds such as pyrrole and pyridine, reactivity with β-propiolactone is influenced by aromaticity. In case of pyrrole, the electron pair on the nitrogen atom is needed to sustain aromaticity. Therefore, this electron pair is less available for the reaction with β-propiolactone. Indeed, no reaction products were observed by NMR when treating an aqueous solution of pyrrole with β-propiolactone. According to theory, pyrrole will react at strong conditions with β-propiolactone at the C-3 carbon atom but not at the nitrogen atom (58). On the other hand, the lone pair on the pyridine nitrogen reacted fairly readily with β-propiolactone, because this electron pair is not involved in the aromaticity of pyridine. Sixty-five percent of the pyridine molecules was converted into N-(2-carboxyethyl) pyridine (NMR: δ 8.88, d, 2H, 8.56, dt, 1H, 8.08, t, 2H, 4.80, t, 2H, 2.92, t, 2H). The proposed structure is given in Table 2. In the case of indole, like pyrrole, no reaction products were observed after treatment of indole with β-propiolactone because the single electron pair of indole is needed for aromaticity (Table 2). As expected, the pyridine-like nitrogen atom N-7 in 7-aza-indole did react with β-propiolactone, whereas the pyrrole-like nitrogen atom N-1 was not modified (Table 2).
The reaction of β-propiolactone with benzimidazole also afforded mono-alkylated (NMR: δ 8.5, s, 1H, 7.78, m, 2H, 7.5, m, 2H, 4.6, t, 2H, 2.81, t, 2H) and bis-alkylated (δ 9.28, s, 1H, 7.91, m, 2H, 7.7, m, 2H, 2.86, t, 2H) products as was observed by NMR. The yields of mono-alkylated and bis-alkylated benzimidazole were high, comparable with that of the alkylated imidazole products (Table 2).

**Aniline, 2-Aminopyridine, and 2-Aminopyrimidine**

Reaction of β-propiolactone with aniline resulted in mono-alkylation (NMR: δ 2.48, t, 2H, 3.38, t, 2H, 6.9, m, 3H, 7.3, m, 2H) and bis-alkylation (NMR: δ 2.55, t, 4H, 3.46, t, 4H, 7.05, m, 3H, 7.4–7.6 m, 2H). The primary amino group of aniline was converted substantially after the reaction, but no acylated product was observed (Table 2). The same products were found after treatment of aniline with 3-iodopropionic acid. 2-Aminopyridine reacted with β-propiolactone to gave two distinct products in a 2:1 ratio (Table 2): an alkylated pyridine nitrogen (NMR: δ 4.39, 2H, t, 2.74, 2H, t) and an alkylated amino group (NMR: δ 3.56, 2H, t, 2.56, 2H, t). 2-Aminopyrimidine reacted with β-propiolactone to give only a pyrimidine-N-alkylated product with a yield of about 12%. The NMR spectrum clearly showed the formation of an unsymmetrical product (NMR: aromatic proton signals at δ 7.1, dd, 1H, 8.38, dd, 1H, and 8.78, 

![FIGURE 2. NMR spectra after the reaction of β-propiolactone (16 mM) in citrate buffer (125 mM, pH 7.8). Spectra were recorded (1–7) after 3, 15, 40, 89, 187, 387, and 775 min. Citrate adducts (C) and 3-hydroxypropionic acid (D) are indicated.](image-url)
Conversion of β-propiolactone in citrate buffer pH 7.8, 125 mM at 25 °C: (Φ) β-propiolactone, (□) 3-hydroxy propionate and (▲) citrate adducts.

dd, 1H, and two aliphatic of CH2 groups at 2.79 and 4.39. No conversion was observed of the primary amino group in 2-aminopyrimidine.

2-Pyridone, Uracil, Pyrimidin-3(4H)-one, and Hypoxanthine

Amide groups that are not nucleophilic do not react with β-propiolactone under conditions tested here. However, it remained uncertain if β-propiolactone can react with the nitrogen atoms of a pyridine ring, such as N-1 of 2-pyridone, N-1 and N-3 of uracil, N-3 of pyrimidin-3(4H)-one, and N-1 of hypoxanthine (Table 2). Therefore, 2-pyridone was treated with β-propiolactone. No reaction products of 2-pyridone were detected by NMR. Also, uracil was not modified by β-propiolactone. In contrast, two alkylated products were formed by the reaction of β-propiolactone with pyrimidin-4(3H)-one (Table 2). The NMR spectrum showed two overlapping triplet signals around 4.2 ppm and two triplets at δ 2.71 and 2.66 ppm, indicative for two distinct NCH2 and CH2CO groups. The total conversion into alkylated nitrogen atoms N-1 and N-3 of pyrimidin-3(4H)-one was 25%. β-Propiolactone reacted with hypoxanthine to give a mixture of alkylated products (8% conversion). The NMR spectrum suggested at least three products; three triplets were observed around δ 4.4 – 4.6 (NCH2) together with three triplets at δ 2.68 and around δ 2.7–2.9 (CH2CO). In the aromatic part of the spectrum, six additional singlets appeared at δ 8.42, 8.35, 8.28, 8.2, 8.15, and 8.11, respectively. The tentative structures are given in Table 2. In conclusion, nitrogen atoms in a pyridone or pyrimidinone ring can react with β-propiolactone, but to a lesser degree than pyridine and pyrimidine.

Reaction of β-Propiolactone with Nucleosides

Based on the reactions with nucleobase analogues, we predicted that position N-7 and N-3 react with β-propiolactone, whereas the nitrogen atom N-1 and the exocyclic nitrogen C2N will probably hardly react.

The nitrogen atoms N-1 and C2N have homology with a pyridone ring or with the primary amino group of 2-aminopyrimidine, respectively. In the case of deoxyguanosine, additional signals were observed for the 1H proton at 6.4 (t, 1H) and the H-8 proton at 8.88 ppm, indicating alkylation at position N-7 (Fig. 5). This product was also identified by Roberts and Warwick (23). At prolonged reaction times, additional H-8 signals at 7.92, 7.96, and 8.00 ppm were observed, indicating an unexpected product derived from the N-7 adduct. The proposed structure of the product is given in Fig. 6 in which the imidazole ring is hydrolyzed. As was shown previously, alkylation at N-7 of guanosine stimulates opening of the imidazole ring during the reaction of chloroethyl ethyl sulfide with 2'-deoxyguanosine (60). No indications were found for modifications at positions N-1, N-3, C6N, and N-7. Only one modification was observed by NMR, consistent with alkylation at N-1, as reported by Chen et al. (61). It showed down-field shifts for the H-2 (8.57, s, 1H) and H-8 (8.50, s, 1H) protons, indicating a charged ring system. In the NMR spectrum (Fig. 5) of the reaction mixture with β-propiolactone and cytidine, new signals were observed at δ 8.1 (d, 1H), 6.29 (d, 1H), and 5.89 (d, 1H). These signals indicate the formation of N-(2-carboxyethyl) cytidine (Table 2). As expected from reactions with nucleobase analogues, deoxythymidine did not react with β-propiolactone. If compared with other studies (23, 51), the reactions were performed with relatively low concentrations of β-propiolactone (16 mM), which might also explain the little number of modifications observed. The concentration of β-propiolactone was comparable with the concentrations normally used for viral inactivation.

Reaction of β-Propiolactone with Peptides

A set of 12 synthetic peptides (Ac-VTLXVTR-NH2, in which X = Ala, Cys, Asp, Glu, His, Lys, Met, Asn, Glu, Ser, Trp, or Tyr) was used to investigate the reactivity of β-propiolactone with specific amino acid residues (Table 3). The reactions were performed in phosphate buffers with a pH of 3, 5, 7, or 9. Peptide 1 was designed to be inert for β-propiolactone. Indeed, LC/MS analyses revealed that peptide 1 was not modified after incubation with β-propiolactone in buffers adjusted to a pH of 3, 5, or 7. However, a minor conversion was observed at pH 9 (Table 3). LC/MS2 analyses showed that peptide 1 gave two products with a mass increment of 72 Da located on either the residue Thr4 or the Thr6 (Fig. 7). Other peptides containing a cysteine (peptide 2), aspartic acid (peptide 3), glutamic acid (peptide 4), lysine (peptide 6), methionine (peptide 7), serine (peptide 10), or tyrosine residue (peptide 12) were found to be modified by β-propiolactone. The conversion of these peptides depended on the pH (Table 3); the higher the pH, the higher the conversion of the peptides, except for the methionine residue. Substantial conversion of the methionine residue was measured at pH 3. This latter result is in agreement with data published previously (52). Peptides containing asparagine, glutamine, or tryptophan residues (peptide 8, 9, 11) did not react with β-propiolactone. Based on the LC/MS analyses, the conversion rate of other amino acid residues was at pH 7 in decreasing order: cysteine > methionine > histidine > aspartic or glutamic acid > tyrosine > lysine > serine > threonine.
Reactions of β-Propiolactone

| Analogue          | Conversion (%) | β-Propiolactone adducts                              |
|-------------------|----------------|-----------------------------------------------------|
| pyrrole           | (0%)           |                                                     |
| pyridine          | (65%)          | 1-(2-carboxyethyl) pyridine                         |
| imidazole         | (50%)          | hydroxypropyl imidazole                             |
|                   |                | 2-carboxyethyl imidazole                            |
|                   |                | 1,3-bis(2-carboxyethyl) imidazole                   |
| aniline           | (40%)          | N-(2-carboxyethyl) aniline                          |
|                   |                | N,N-di(2-carboxyethyl) aniline                      |
| 2-aminopyridine   | (15%)          | 1-(2-carboxyethyl) 2-aminopyridine                  |
|                   |                | N-(2-carboxyethyl) 2-aminopyridine                  |
| 2-aminopirimidine | (12%)          | 1-(2-carboxyethyl) 2-aminopirimidine                |
| indole            | (0%)           |                                                     |
| 7-azaindole       | (35%)          | 7-(2-carboxyethyl) azaindole                        |
| benzimidazole     | (30%)          | 1-(2-carboxyethyl) benzimidazole                    |
|                   |                | 1,3-bis(2-carboxyethyl) benzimidazole               |
| 2-pyridone        | (0%)           |                                                     |
| pyrimidin-4(3H)-one | (25%)      | 1-(2-carboxyethyl) 4-hydroxyimididine               |
|                   |                | 3-(2-carboxyethyl) 4-hydroxyimididine               |
The treatment of cysteine residues (peptide 2) with \( \text{H}_9\text{R}52\text{-propiolactone} \) resulted in four reaction products that could be separated chromatographically (Fig. 8). LC/MS revealed two adducts with a mass increment of 72 Da (product I and II) and two adducts with an increase of 144 Da (product III and IV). LC/MS2 analysis was used to verify that adducts of \( \text{H}_9\text{R}52\text{-propiolactone} \) were attached to cysteine residue, although the interpretation of MS2 spectra is rather complex (Fig. 8). Fragmentation of products I and II started with a characteristic neutral loss of 106 Da (C\(_3\)H\(_6\)O\(_2\)S) followed by the fragmentation of the peptide backbone (Table 4). The neutral loss resulted in a truncated cysteine side chain (\( \Delta M = 72\text{–}106 \) Da). MS2 spectra obtained from products I and II are comparable, except for the amount the neutral loss fragment formed (neutral loss of product I > product II). MS2 analysis of product III and IV containing \( \text{H}_9\text{R}52\text{-propiolactone} \) adducts resulted also in fragments with neutral losses. The spectrum of product III showed a fragment with neutral loss of 178 Da. Neutral loss of 178 Da can be explained by truncation of the cysteine residue (C\(_6\)H\(_{10}\)O\(_4\)S). However, inadequate backbone fragmentation occurred. The spectrum of product IV demonstrated neutral losses of 72 Da (C\(_2\)H\(_4\)O\(_2\)) and 178 Da (C\(_6\)H\(_{10}\)O\(_4\)S), and also, backbone fragmentation occurred.

The two products with a mass increment of 72 Da can be explained as acylation (product I) and alkylation (product II) of
the cysteine residue. The assignment of both peaks was based on the results obtained from the reaction of peptide 2 with 3-iodopropionic acid. This compound can only alkylate reactive amino acid residues. Alkylated products of /H9252-propiolactone and 3-iodopropionic acid have the same structure. As expected treatment with 3-iodopropionic acid resulted in only one product with a mass increment of 72 Da. The retention time of this product was comparable with product II of the peptide 2 treated with /H9252-propiolactone. MS2 analysis confirmed that the cysteine residue of peptide 2 was modified by 3-iodopropionic acid, and the MS2 spectrum was exactly the same as the MS2 spectrum of the product in product II (Fig. 8). In addition, the treatment of peptide 2 with 3-iodopropionic acid gave two products with an increase of 144 Da. The retention times on the column, mass increments, and MS2 spectra of these two products were the same as for products III and IV from β-propiolactone-treated peptide 2. A mixture of β-propiolactone and 3-iodopropionic acid-treated peptide 2 was prepared (in 1:1 molar ratio) and analyzed by LC/MS. The measurement confirmed that the products II-IV were identical because of the elution times and masses (supplemental Fig. S1).

In a different experiment, the reaction of β-propiolactone with a cysteine analog (Ac-Cys-OMe) was monitored by NMR. The analysis revealed that cysteine reacts very fast with β-propiolactone at a pH of 9.4 (bicarbonate buffer 100 mM) to give both alkylated (NMR: δ 2.07, 3H, s, 2.47, 2H, t, 2.79, 2H, t, 2.95–3.12, m, 2H, 3.79, 3H, s) and acylated (NMR: δ 2.03, 3H, s, 2.9, 2H, t, 3.26–3.53, 2H, m, 3.78, 3H, s, 3.89, 2H, t) products in about a 2:1 ratio, respectively (Table 5). The formation of acylated product is somewhat unexpected, because Hard Soft Acids Bases theory predicts alkylated product (the thiol group is considered to be a prototypically soft nucleophile (47)). When a 10-fold excess of β-propiolactone was used, bis-alkylated product was also formed. The proposed structures of modified cysteine residues are given in Table 5.

Cystine—According to the literature, β-propiolactone reacts also with disulfide groups (62–64). However, this finding could not be supported in the present study. Peptide 2 containing a cysteine residue was treated with iron(III) chloride to oxidize peptide 2. LC/MS analysis revealed that the oxidation resulted in the formation of a disulfide bridge between two peptide molecules (product MH+ = 1661.92 ± 0.02 Da). Based on the peak areas, 97% of peptide 2 was converted into a dipeptide containing a disulfide bridge. Subsequently, the oxidized peptide was treated with β-propiolactone. The expected products (MH+ = 1733.9 and 1806.0 Da) were not detected by LC/MS, whereas the residual amount of peptide 2 (thiol) was substantially converted by β-propiolactone (MH+ = 904.5 Da). A second experiment confirmed that disulfide groups do not react with β-propiolactone. A cystine derivative (Ac-Cys-OMe)2 was treated with β-propiolactone and monitored by NMR at regular intervals. No reaction products were generated. We conclude that cystine residues do not react with β-propiolactone.
Reactions of β-Propiolactone

Aspartic and Glutamic Acid—The reaction of β-propiolactone with peptides containing an aspartic or glutamic acid residue (peptides 3 and 4, respectively) resulted in a major product with a mass increment of 72 Da and a minor component with an increase of 144 Da (conversions of about 8 and 0.1%, respectively). LC/MS² analyses confirmed that one or two β-propiolactone molecules was attached to the aspartic or glutamic acid residue (supplemental Fig. S2). The same products were obtained, albeit in low yields by treating peptides 3 and 4 with 3-iodopropionic acid. Retention times, mass increments, and MS² spectra were identical for either the β-propiolactone- or 3-iodopropionic acid-modified peptides (peptides 3 or 4).

Based on these results, we conclude that aspartic or glutamic acid residues are probably alkylated by the reaction with β-propiolactone. The proposed structures of β-propiolactone-modified aspartic or glutamic acid residues are given in Table 5.

Histidine—Two reaction products were found after incubation of a peptide 5 (containing a histidine residue) with β-propiolactone. Two products of peptide 5 were chromatographically separated and detected in significant amounts by LC/MS with a mass increment of 72 and 144 Da (Fig. 9).

Two distinct products with a mass increase of 72 Da were expected, because it has been postulated that histidine can be alkylated at position N-1 or at position N-3 of the imidazole group (65). However LC/MS revealed only one chromatographically separated product with a mass increment of 72 Da. If histidines were alkylated at position N-1 or at position N-3, the peptides eluted simultaneously.

As LC/MS analysis showed that β-propiolactone (ΔM of 72 Da) was present at the histidine residue (Fig. 9). Unfortunately, LC/MS analysis cannot reveal which nitrogen of the histidine residue is modified: position N-1 and/or N-3. The product with mass increase of 144 Da showed poor fragmentation during MS² analysis. Observed neutral loss fragments of 17, 44, and 61 Da were ascribed to truncation of the arginine side chain.

Multiple activation performed on fragment ions revealed the peptide sequence and the modified histidine residue (Fig. 9). Treatment of peptide 5 with 3-iodopropionic acid did not result in significant conversion of the histidine residue. The experiment with 3-iodopropionic acid did not support that alkylation of

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**TABLE 3**

Peptides treated with β-propiolactone at different pH values

| Peptide | Sequence | Mass | Adducts | ΔM | Conversion |
|---------|----------|------|---------|----|-----------|
| 1       | Ac-VTLAVTR-NH₂ | 799.492 | 0⁴ | - | pH 3.0 |
| 2       | Ac-VTLAVTR-NH₂ | 831.464 | 4 | 72/144 | - |
| 3       | Ac-VTLAVTR-NH₂ | 843.481 | 2 | 72/144 | 1.8 ± 0.3 |
| 4       | Ac-VTLAVTR-NH₂ | 857.497 | 2 | 72/144 | 0.5 ± 0.1 |
| 5       | Ac-VTLAVTR-NH₂ | 865.513 | 2 | 72/144 | 0.3 ± 0.0 |
| 6       | Ac-VTLAVTR-NH₂ | 856.549 | 0 | 72/144 | 0.7 ± 0.2 |
| 7       | Ac-VTLAVTR-NH₂ | 849.509 | 2 | 72/144 | 58.7 ± 2.8 |
| 8       | Ac-VTLAVTR-NH₂ | 842.497 | 0 | - | - |
| 9       | Ac-VTLAVTR-NH₂ | 856.513 | 0 | - | - |
| 10      | Ac-VTLAVTR-NH₂ | 845.487 | 0 | - | - |
| 11      | Ac-VTLAVTR-NH₂ | 891.518 | 1 | - | - |
| 12      | Ac-VTLAVTR-NH₂ | 891.518 | 1 | - | - |

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⁴ The number of β-propiolactone adducts attached to the residue in bold (X) are given in bold. Reaction products were identified based on the number of peaks in HPLC chromatograms and MS² data.

⁵ Data are the mean ± S.D.; n = 3.

⁶ The alanine residue is not modified. However, two reaction products were found in which one of the threonine residues was slightly modified by β-propiolactone at a pH of 9.0.

⁷ No reaction products of β-propiolactone were detected.

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**FIGURE 7.** LC/MS chromatograms and MS² spectra were obtained from peptide 1 after treatment with β-propiolactone at pH of 9.0. Peptide 1 was slightly converted in two reaction products with a β-propiolactone attached to Thr² (I) and Thr² (II) residues, resulting in a mass increment of 72 Da. MS² spectra revealed also a characteristic fragment with a neutral loss of 90 Da (C₇H₁₀O₇).
Reactions of β-Propiolactone

Ion trace 832.47 Da, I = 2.3E5

Ion trace 904.49 Da, I = 5.3E6

Ion trace 976.51 Da, I = 6.6E5

Time (min)
25.0  26.0  27.0  28.0  29.0  30.0

m/z
300  400  500  600  700  800  900  1000

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histidine residues occurs during the reaction with β-propiolactone. In addition, the reaction of a histidine derivative (Ac-His-NHMMe) with β-propiolactone was recorded by NMR. The reaction resulted in mono-alkylation and bis-alkylation (NMR: δ 6.8, s, 1H, 7.6, s, 1H and 7.3, s, 1H, 7.7, s, 1H, respectively). Also, NMR demonstrated the presence of only one monoalkylated product, which is in accordance with the results obtained from LC/MS analysis. Because the nitrogen atom N-1 of histidine is less accessible than the nitrogen atom N-3, we assume that the histidine residues will be monoalkylated at position N-3. The proposed structures of β-propiolactone-modified histidine residues are given in Table 5.

**Lysine**—Two reaction products with only one attachment of β-propiolactone (ΔM of 72 Da) were detected on peptide 6. Both products were widely separated by chromatography (Fig. 10). MS² measurement confirmed the modification of the lysine residue. The two products can be explained by alkylation and acylation reactions of β-propiolactone (Table 5). Both structures were described previously as N-(2-carboxyethyl)lysine and N-(3-hydroxypropionyl)lysine (66). Alkylation of the lysine side chain forms a secondary amine group that is protonated at a low pH. The polarity of the modified peptide and retention times on the column will not change drastically if compared with the non-modified peptide. Therefore, product I is probably peptide 6 with an alkylated lysine residue (Fig. 10), because the peptide was detected as a double-charged ion with a slightly longer retention time (Δt = ~0.5 min). In the case of acylation of lysine residues, an amide (peptide) bond is formed that resulted in neutral moiety at a low pH. Therefore, acylation of peptides reduces the polarity and increases the retention times. Indeed, product II mainly consist of single-charged ions with a prolonged retention time (Δt = ~2.5 min). The reaction of β-propiolactone with a lysine derivative (Ac-Lys-OMe) resulted in both acylated and alkylated products (Table 5). The alkylated product showed characteristic triplets for the secondary amine group (–CH₂NHCH₂–) at δ 3.18 and 3.20 ppm, whereas the acylated product gave triplets for -N(CO)CH₂- at δ 2.57 and 3.84 ppm.

**Methionine**—LC/MS analysis revealed two adducts with one or two β-propiolactone molecules attached to peptide 7 (Fig. 11). The product with a single attachment of β-propiolactone (ΔM of 72 Da) eluted 5 min earlier than the native peptide, indicating strongly increased polarity. The adduct containing two β-propiolactone molecules (ΔM of 144 Da) eluted 0.5 min later than the product with a single attachment. The structure of a single attachment to methionine has been described in the literature (52) and explains the increased polarity due to the positively charged sulfur atom. MS² measurement did not provide any sequence information. Modified methionines present in peptides resulted in particular neutral losses of 120 Da for the single attachment (C₄H₈O₂S) and 192 Da for the double attachment of β-propiolactone (C₆H₁₄O₄S). However, multistage activation on the peptide fragment with a neutral loss (MH⁺ or of 406.8) provided the full peptide sequence (Fig. 11) with a truncated methionine side chain (ΔM of ~48 Da = 72–120 Da). The reaction of 3-iodopropionic acid with peptide 7 resulted in two reaction products with the same mass increments, retention times, and MS² spectra as the products of β-propiolactone-treated peptide 7, indicating that β-propiolactone only alkylates methionine residues. A mixture of β-propiolactone and 3-iodopropionic acid-treated peptide 7 (in 1:1 ratio) demonstrated by LC/MS the same elution times and masses for the products I and II (supplemental Fig. S3). NMR analysis of Ac-Met-OMe treated with β-propiolactone revealed that an alkylated product was formed (Table 5). In the NMR spectrum, two characteristic singlets were observed corresponding to the diastereomers (around 2.95 ppm) of the S-methyl group together with additional signals of the alkylated product (NMR: δ 4.65, m, 1H, 3.58–3.32, m, 3.18 and 3.20 ppm).

**Serine**—In addition to LC/MS analysis performed on peptide 2 treated with β-propiolactone, four products (I–IV) were identified by LC/MS in which the cysteine residue was modified by β-propiolactone. MS² spectra I–IV revealed the peptide sequence with a truncated cysteine side chain (–CH₂SH–) (Fig. 12). Fragmentation during MS² analysis resulted in a neutral loss fragment of 106 Da (C₄H₈O₂S) or 178 Da (C₆H₁₀O₄S) if one or two β-propiolactone molecules were attached to the cysteine residue respectively.

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**TABLE 4**

Typical neutral loss fragments of β-propiolactone-modified amino acid residues

| Residue | Product | ΔM | Neutral loss fragments |
|---------|---------|----|-----------------------|
| Cysteine| I       | 106| HS-CO-CH₂-CH₂OH       |
|         | II      | 106| HS-CO-CH₂-CH₂-CH₂COOH |
|         | III     | 178| HOOC-CH₂-CH₂-S-CH₂-CH₂COOH |
|         | IV      | 72 | H₂C=CH-COOG           |
|         |         |    | C₂H₂O₂S               |
| Aspartate| I      | 72 | H₂C=CH-COOG           |
|         | II      | 72 | H₂C=CH-COOG           |
|         |         |    | C₂H₂O₂S               |
| glutamate| I      | 144| H₂C=CH-COOG           |
|         | II      | 144| H₂C=CH-COOG           |
|         |         |    | C₂H₂O₂S               |
| Histidine| -      | -  | -                      |
| Lysine  | I       | 120| H₂C-S-CH₂-CH₂-COOH    |
|         | II      | 192| H₂C-S-CH₂-CH₂-(CO)-O-CH₂-CH₂-COOH |
|         |         |    | C₄H₈O₄S               |
| Methionine| I     | 90 | HO-CH₂-CH₂-COOH       |
|         | II      | 90 | HO-CH₂-CH₂-COOH       |
|         |         |    | C₂H₂O₂S               |

* No neutral loss fragments were detected.

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**FIGURE 8.** LC/MS analyses performed on peptide 2 treated with β-propiolactone. Four products (I–IV) were identified by LC/MS in which the cysteine residue was modified by β-propiolactone. MS² spectra I–IV revealed the peptide sequence with a truncated cysteine side chain (–CH₂SH–) (Fig. 12). Fragmentation during MS² analysis resulted in a neutral loss fragment of 106 Da (C₄H₈O₂S) or 178 Da (C₆H₁₀O₄S) if one or two β-propiolactone molecules were attached to the cysteine residue respectively.

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**FIGURE 10.** NMR analysis of Ac-Met-OMe treated with β-propiolactone revealed that an alkylated product was formed (Table 5). In the NMR spectrum, two characteristic singlets were observed corresponding to the diastereomers (around 2.95 ppm) of the S-methyl group together with additional signals of the alkylated product (NMR: δ 4.65, m, 1H, 3.58–3.32, m, 3.18 and 3.20 ppm).
### Proposed structures of β-propiolactone-modified amino acid residues

| Residue | β-Propiolactone adducts |
|---------|-------------------------|
| Cysteine (C) | ![Structure](image1) |
| | +72 Da 3-hydroxypropionyl cysteine |
| | n x +72 Da (n=1,2) |
| | n1: 2-carboxyethyl cysteine |
| | n2: 2-carboxyethyl(2-carboxyethyl) cysteine |
| | +144 Da bis(2-carboxyethyl) cysteine |
| Aspartic acid (D) / Glutamic acid (E) | ![Structure](image2) |
| | D: m1=1; E: m2=2 |
| | n x +72 Da (n=1,2) |
| | m1: 2-carboxyethyl aspartate |
| | m2: 2-carboxyethyl glutamate |
| Histidine (H) | ![Structure](image3) |
| | +72 Da 3-(2-carboxyethyl) histidine |
| | 1,3-bis(2-carboxyethyl) histidine |
| Lysine (K) | ![Structure](image4) |
| | +72 Da 3-hydroxypropionyl lysine |
| | 2-carboxyethyl lysine |
| Methionine (M) | ![Structure](image5) |
| | n x +72 Da (n=1,2) |
| | m1: 2-carboxyethyl methionine |
| | m2: 2-carboxyethyl(2-carboxyethyl) methionine |
| Serine (S) / Threonine (T) | ![Structure](image6) |
| | S: R''=H; T: R''=CH3 |
| | +72 Da |
| | S: 3-hydroxypropionyl serine |
| | T: 3-hydroxypropionyl threonine |
| Tyrosine (Y) | ![Structure](image7) |
| | +72 Da 3-hydroxypropionyl tyrosine |
When the reaction was carried out with a 10-fold excess of β-propiolactone, another sulfonium adduct was observed (NMR: δ 2.97, ds, 3H, 3.04, dt, 2H, 4.41, t, 2H). The NMR data obtained are in accordance with a biscarboxyethoxylated methionine. Both MS and NMR analyses confirmed that methionine residues become alkylated by the reaction of β-propiolactone. The proposed structures of both products are given in Table 5.
Serine and Tyrosine—Peptides containing a serine or tyrosine residue (peptide 10 and 12, respectively) were slightly converted at pH 7 to a product with a mass increment of 72 Da, corresponding to a single attachment of \( \text{H}9252 \text{-propiolactone} \) (Table 5). MS\(^2 \) analysis confirmed that \( \text{H}9252 \text{-propiolactone} \) was attached to the serine or tyrosine residue (supplemental Figs. 4 and 5). \( \beta \)-Propiolactone probably reacts with the hydroxyl group of serine or tyrosine. The MS\(^2 \) spectrum of the peptide 10 with a modified serine residue showed a typical fragment ion with a neutral loss of 90 Da \( (\text{C}_3\text{H}_6\text{O}_3) \). A fragment ion with a similar neutral loss of 90 Da was not observed for peptide 12 with a modified tyrosine residue (Table 4).

From the literature it remains unclear if serine, threonine, and tyrosine residues are acylated or alkylated by \( \text{H}9252 \text{-propiolactone} \). Determann and Joachim (67) found only an acid labile product from tyrosine and concluded that acylation occurred.
were acylated by sine residues were completely hydrolyzed, indicating that they
sequence with a truncated methionine residue (activation performed on the fragment ion (406.8) revealed the peptide
vided only a fragment ion (406.8) with a neutral loss of 120 Da.

Theory 7. Theoretical predictions of the serine and tyrosine residues. In addition, a tyrosine
were observed for cysteine and methionine residues. Less, but significant, conversions were determined for the nucleosides
deoxyadenosine, cytidine, and deoxyguanosine. The results indicate that proteins are more extensively modified by than
nucleic acids during inactivation of viruses with \( \beta \)-propiolactone. The amount and nature of modifications in viral compo-
nents, i.e. proteins, DNA, or RNA, will be dependent on con-

This was in contrast with results obtained by Gresham et al. (68). They observed alkylation of the phenol during the reaction
with \( \beta \)-propiolactone, although acylation of the phenol occurred under acid catalysis. Also, theoretical predictions
made by Zhang and Yang (69) indicate that alkylation of tyro-
sine residue will occur by treatment of

FIGURE 11. LC/MS analyses performed on \( \beta \)-propiolactone-treated pep-
tide 7. LC/MS chromatograms demonstrated two reaction products (I and II) with a single and double \( \beta \)-propiolactone attachment. Ia, the MS\(^2\) spectrum obtained from peptide 7 with a single attachment of \( \beta \)-propiolactone pro-
vided only a fragment ion (406.8) with a neutral loss of 120 Da. Iib, multistage activation performed on the fragment ion (406.8) revealed the peptide
sequence with a truncated methionine residue (48 Da). The second product (II) with two attachments of \( \beta \)-propiolactone resulted in a comparable (Ia) MS\(^2\) spectrum and (Ib) multistage fragmentation spectrum.

\( \beta \)-Propiolactone is extensively used for the production of inactivated viral vaccines against influenza and rabies. Despite decades of large scale use of these vaccines, the chemical mod-
ifications that occur during viral inactivation were only partially
known. Here we present a detailed and systematic overview of \( \beta \)-propiolactone modifications on the building blocks of
nucleic acids and proteins: nucleobase analogues, nucleosides, and amino acid residues. The nucleosides deoxyadenyline, cyto-
sine, and deoxyguanine were modified by \( \beta \)-propiolactone, alkylation on positions N-1 of deoxyadenyline, on exocyclic amino group of cytosine, and on position N-7 of deoxyguanine. Furthermore, nine amino acid residues were shown to react with \( \beta \)-propiolactone (Table 3). In many cases the amino acid residues were alkylated, but serine, threonine, and tyrosine res-
ides were exclusively acylated. Cysteine and lysine residues were partially acylated and alkylated. Unexpectedly, it was
shown that disulfide groups in cystine residues do not react with \( \beta \)-propiolactone. On the other hand, highest conversions were observed for cysteine and methionine residues. Less,

Conclusions

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In this study the relative reactivity of nucleosides and amino
acid residues for \( \beta \)-propiolactone was elucidated. The data can be utilized to predict or to elucidate through LC/MS or NMR studies the chemical modifications occurring in viral compo-
nents during the inactivation of viruses with \( \beta \)-propiolactone.

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