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Mild and convenient *N*-formylation protocol in water-containing solvents

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Abstract

We have realized that *N*-formylations of free amines of some drug leads can improve PK/PD property of parent molecules without decreasing their biological activities. In order to selectively formylate *primary* amines of polyfunctional molecules, we have sought a mild and convenient formylation reaction. In our screening of *N*-formylation of an α-amino acid, L-phenylalanine, none of formylation conditions reported to date yielded the desired HCO-L-Phe-OH with satisfactory yield. *N*-Formylations of amino acids with HCO_2H require the reactions in a water-containing media and suppress polymerization reactions due to the competitive reactions among carboxylic acids. We found that *N*-formylations of α-amino acids could be achieved with a water-soluble peptide coupling additive, an oxyma derivative, (2,2-dimethyl-1,3-dioxolan-4-yl)methyl-2-cyano-2-(hydroxyimino)acetate (**2**), EDCI, and $NaHCO_3$ in water or a mixture of water and DMF system, yielding *N*-formylated α-amino acids with excellent yields. Moreover, these conditions could selectively formylate *primary* amines over *secondary* amines at a controlled temperature. A usefulness of these conditions was demonstrated by selective formylation of daptomycin antibiotic which contains three different amino groups.

Keywords

N-formylation; Reactions in water media; Water-soluble oxyma; Glyceroacetonide-oxyma; Amino acids; Kanamycin; Spectinomycin; Daptomycin

1. Introduction

In our SAR studies of antibacterial agents, we have realized that *N*-formylations of free amines of some antibiotics do not significantly decrease their bioactivities and can be applied to improve PK/PD property of parental molecules. Because of necessity of selective formylation reactions of antibiotics and antibacterial agents in our ongoing programs, we have sought a mild and convenient *N*-formylation reaction condition that can be applied to a wide range of complex natural products, oligo- to poly-peptides, and amino acids. To date, the numerous formylating agents and conditions have been reported.[1] Although several formylating agents can be applicable for the formylations of *C*-protected amino acids, it is not possible to achieve effective formylation reactions for non-protected amino acids with reported reagents and conditions.[2] In addition, many formylating agents are hygroscopic and are not tolerated in appropriate solvents for the reactions for amino acids and oligo-peptides (e.g. water-containing solvents). In our recent finding of amide-forming reactions with the ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma, **1**) derivative, glyceroacetonide-Oxyma **2**

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in water media (Figure 1),[3] it was observed that formylation of H-L-Phe-OH could be achieved with HCO_2H (5 eq.), **2** (2 eq.), EDCI (2 eq.) and $NaHCO_3$ (10 eq.) in water (0.2–0.3 M) to yield the corresponding HCO-L-Phe-OH in greater than 90% yield. On the other hand, the same reaction in the absence of glyceroacetonide-Oxyma **2** did not furnish the desired HCO-L-Phe-OH. Thus, effectiveness of glyceroacetonide-Oxyma **2** in the formylation of amino acid in water was unambiguously determined. Herein, we report mild and convenient *N*-formylations in water or water-containing solvent systems, and selective *N*-formylations of *primary* amines.

2. Results and discussion

Formylation of H-L-Phe-OH with HCO_2H, glyceroacetonide-Oxyma **2**, EDCI, $NaHCO_3$ in water seems to undergo through the well-known reaction mechanism with EDCI,[4] however, in this reaction several interesting chemical observations are worth mentioning. HCO_2H reacts with EDCI faster than H-L-Phe-OH; 5 equivalent of HCO_2H could completely suppress the undesired competitive reaction with H-L-Phe-OH. Due to the fact that formylation of H-L-Phe-OH with EDCI in water did not proceed in the absence of **2**, the initial intermediate, carbamimidic formic anhydride **3** may have a relatively short half-life or not be a good electrophile as a formylating agent in water. However, the intermediate **3** reacts with the glyceroacetonide-Oxyma **2**-sodium salt[5] to furnish the active ester **4** which has a relatively long half-life and serves as *N*-formylating agent in water. It is important to note that formylation of H-L-Phe-OH with Oxyma **1** in water furnished the desired product in very low yield (<10%). As observed in peptide-forming reactions, formylation using **1** could be improved dramatically when the reaction was performed in a mixture of DMF-H_2O (9/1).[3b] Thus, **1** and **2** can efficiently be utilized for formylation of H-L-Phe-OH by using water or a mixture of water and DMF. However, glyceroacetonide-Oxyma **2** has a significant advantage over **1** in that **2** can be removed completely after the reactions via an acidic water work-up, thus, the only formylated-products can be extracted from reaction mixtures after a simple work-up.

In order to examine scope and limitations of *N*-formylation reactions with HCO_2H, **2** (or **1**), EDCI, and $NaHCO_3$ in H_2O (condition **A**) or in DMF-H_2O (9/1, condition **B**), we have applied these conditions to a wide variety of *primary* and *secondary* amines, and α-amino acids. As observed for H-L-Phe-OH, formylations of all α-amino acids tested in this program provided the corresponding *N*-formylated products in H_2O. Representative data are summarized in Table 1 (entries 15–18). In all cases *N*-formylations of α-amino acids with condition **A** furnished the desired products in better yield than those with condition **B** (85–95 vs 30–60% yield). We have demonstrated *N*-formylation of an oligopeptide in water; *N*-formylation of the pentapeptide with condition **A** yielded the corresponding formylation product in 90% (entry 19). *N*-Formylation of *C*-protected α-amino acids could be achieved efficiently either with condition **A** or **B** without noticeable difference in yield of the products (entries 8–10). Thus, formylations of aliphatic and aromatic amines were performed with Oxyma **1** in DMF-H_2O (condition **B**); *N*-formylations of benzylamine, octylamine, and aniline provided the corresponding products in quantitative yield (entries 1, 2, and 5). *N*-Formylation reactions of a monoprotected 1,3-diamine and an amino-alcohol provided the *N*-formylated products in excellent yields (entries 3 and 4). On the other hand, *N*-formylations of 2-aminobenzoic acid and 2-aminophenol gave rise to the desired products in 30% and 25% yield, respectively (entries 6 and 7).[6] Formylations of *secondary* amines, piperidine, morpholine, L-Pro-OMe, and *N*-Me-L-Val-OMe were completed within 3h to yield the corresponding products in good yields (entries 11, 12, 13, and 14). Interestingly, formylation of a *secondary* amine, *N*-Me-L-Val-OMe provided the formylated-product in less than 5% yield at 0 °C, whereas a *primary* amine H-L-Val-OCH_3 was formylated at 0 °C-rt. The rate of the reaction progress of formylations of *N*-Me-L-Val-OMe and H-L-Val-

OCH_3 in H_2O (condition **A**) was monitored over time and their reaction kinetic curves are shown in Figure 2. The striking difference in reaction rate for formylations of *primary* and *secondary* amines was observed when the reactions were performed in water or in water-containing solvents.

We have applied these formylation reaction conditions to several antibacterial natural products. Selective *N*-formylation of kanamycin A could be achieved at the *primary* amine, yielding the 6′-formylated kanamycin A in 30% isolation yield (65% yield based on LC-MS) (entry 20 in Table 1).[7] Formylation of spectinomycin in H_2O at rt furnished the mono-formylated product in 50% yield (entry 21).[8] Daptomycin is a cyclic lipopeptide antibiotic used in the treatment of certain community-associated methicillin resistant *S. aureus* (CA-MRSA) and healthcare-associated-MRSA (HA-MRSA) infections.[9] Daptomycin possesses stereoelectronically different three free amines, four carboxylic acids, a free alcohol in the molecule, however, shows limited water solubility. Selective *N*-formylation of daptomycin was achieved at the *primary* amine of the lysine residue in DMF-H_2O (2/1) to provide the expected *N*-formylation product in 65% isolation yield after a reverse HPLC purification (90% yield based on analysis of the crude product via ^{1}H-NMR and LC-MS) (Scheme 2).[10]

In summary, we have demonstrated selective *N*-formylation reactions using HCO_2H, Oxyma **1** or glyceroacetonide-Oxyma **2**, EDCI, and $NaHCO_3$ in DMF-H_2O system or in H_2O.[11] The *N*-formylation reaction conditions described here do not require strict anhydrous conditions necessary for ordinal formylation reactions.[1,2] To the best of our knowledge, *N*-formylation reactions of α-amino acids have never been achieved efficiently without a suitable *C*-protection. We demonstrated that high yielding *N*-formylations of α-amino acids could readily be accomplished with the described conditions. Glyceroacetonide-Oxyma **2** displays remarkable physico-chemical properties as an additive of *N*-formylation reactions with EDCI in water media. Importantly, simple aqueous work-up procedures can remove all reagents utilized in the reactions to afford *N*-formylation products in high yield with excellent purity.

Acknowledgments

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References and notes

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5. We have demonstrated that Oxyma **1**and glyceroacetonide-Oxyma **2**exist as their Na salts in aq. $NaHCO_3$ solution.

6. Poor reactivity of 2-aminobenzoic acid and 2-aminophenol in these formylations is probably due to the strong formation of intramolecular hydrogen bonding between the NH_2 and COOH or OH groups.

7. Difference in reactivity of the nitrogen atoms in kanamycin and amikacin, see Hanessian S, Kornienkoa A, Swayze EE. Tetrahedron. 2003; 59:995.Bera S, Zhanel GG, Schweizer F. J Med Chem. 2010; 53:3626. [PubMed: 20373816] Kawaguchi H, Naito T, Nakagawa S, Fujisawa K. Antibiotics. 1972; 25:695.Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM. Antimicrob Agents Chemother. 1999; 43:727. [PubMed: 10103173] Mingeot-Leclercq MP, Tulkens PM. Antimicrob Agents Chemother. 1999; 43:1003. [PubMed: 10223907]

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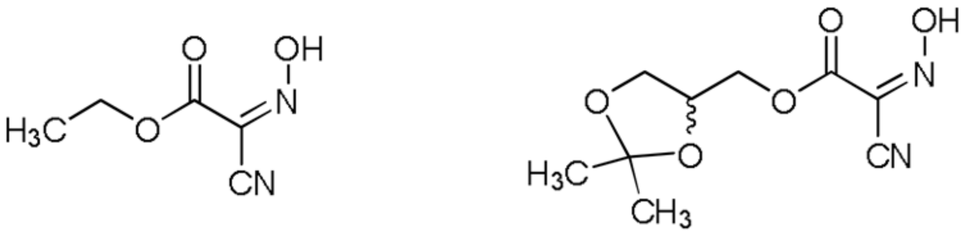
10. $[\alpha]_D^{23}$ = +30 °(*c* 0.1, $CHCl_3$); IR (neat) 3302, 3063, 2928, 2856, 1723, 1717, 1657, 1545, 1536, 1503, 1454, 1408, 1203, 1142, 1024, 828, 742 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 12.31 (s, 4H), 10.80 (d, *J* = 2.4 Hz, 1H), 8.51–8.43 (m, 2H), 8.37 (d, *J* = 7.6 Hz, 3H), 8.26 (t, *J* = 6.1 Hz, 1H), 8.16 (d, *J* = 7.4 Hz, 3H), 8.07 (d, *J* = 5.7 Hz, 1H), 8.03 (d, *J* = 6.3 Hz, 1H), 8.02 (d, *J* = 1.7 Hz, 1H), 7.96–7.91 (m, 1H), 7.77 (t, *J* = 9.1 Hz, 2H), 7.69–7.57 (m, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 7.30–7.25 (m, 1H), 7.18 (dd, *J* = 26.7, 24.4 Hz, 2H), 7.10–7.05 (m, 1H), 7.01–6.97 (m, 1H), 6.92 (s, 1H), 6.77 (d, *J* = 7.9 Hz, 1H), 6.56 (t, *J* = 7.6 Hz, 1H), 5.12–5.04 (m, 1H), 4.92–4.84 (m, 1H), 4.70–4.47 (m, 8H), 4.46–4.40 (m, 1H), 4.31–4.24 (m, 1H), 4.18–4.08 (m, 2H), 3.87 (d, *J* = 13.9 Hz, 1H), 3.49–3.42 (m, 2H), 3.21 (s, 1H), 3.15–3.04 (m, 3H), 2.94 (dd, *J* = 14.8, 9.0 Hz, 1H), 2.80 (ddd, *J* = 27.9, 16.7, 5.6 Hz, 2H), 2.68–2.57 (m, 2H), 2.51–2.39 (m, 4H), 2.37 (p, *J* = 1.9 Hz, 1H), 2.35–2.26 (m, 2H), 2.06 (t, *J* = 7.3 Hz, 2H), 1.94 (dd, *J* = 15.6, 10.2 Hz, 1H), 1.78–1.66 (m, 1H), 1.61–1.44 (m, 3H), 1.43–1.34 (m, 2H), 1.30–1.21 (m, 10H), 1.21–1.14 (m, 5H), 1.11 (d, *J* = 6.4 Hz, 6H), 0.87 (q, *J* = 6.9 Hz, 6H); HRMS (EI) calcd for $C_{73}H_{102}N_{17}O_{27}$ (M + H^+): 1648.7131, found: 1648.7135.

11. General procedure for *N*-formylations: To a solution of amine (1 eq.), formic acid (5 eq.), sodium bicarbonate (10 eq.), and glyceroacetonide-Oxyma **1** (2 eq.) in H_2O (0.2–0.3M) solution was added EDCI (2 eq.) The reaction mixture was stirred for 3h and quenched with 1% aq. HCl. The aqueous phase was extracted with EtOAc (or $CHCl_3$ or $CHCl_3$-MeOH (10/1). The combined organic extracts were dried over Na_2SO_4 and evaporated *in vacuo*. Purification by a silica gel

chromatography (or sephadex LH20) afforded the desired compound (yields were given in Table 1). Similarly, *N*-formylations were performed with Oxyma **1**in DMF-H_2O (9/1).



1: Oxyma **2**: Glyceroacetonide-Oxyma

Figure 1.

Structures of Oxyma **1** and glyceroacetonide-Oxyma **2**.

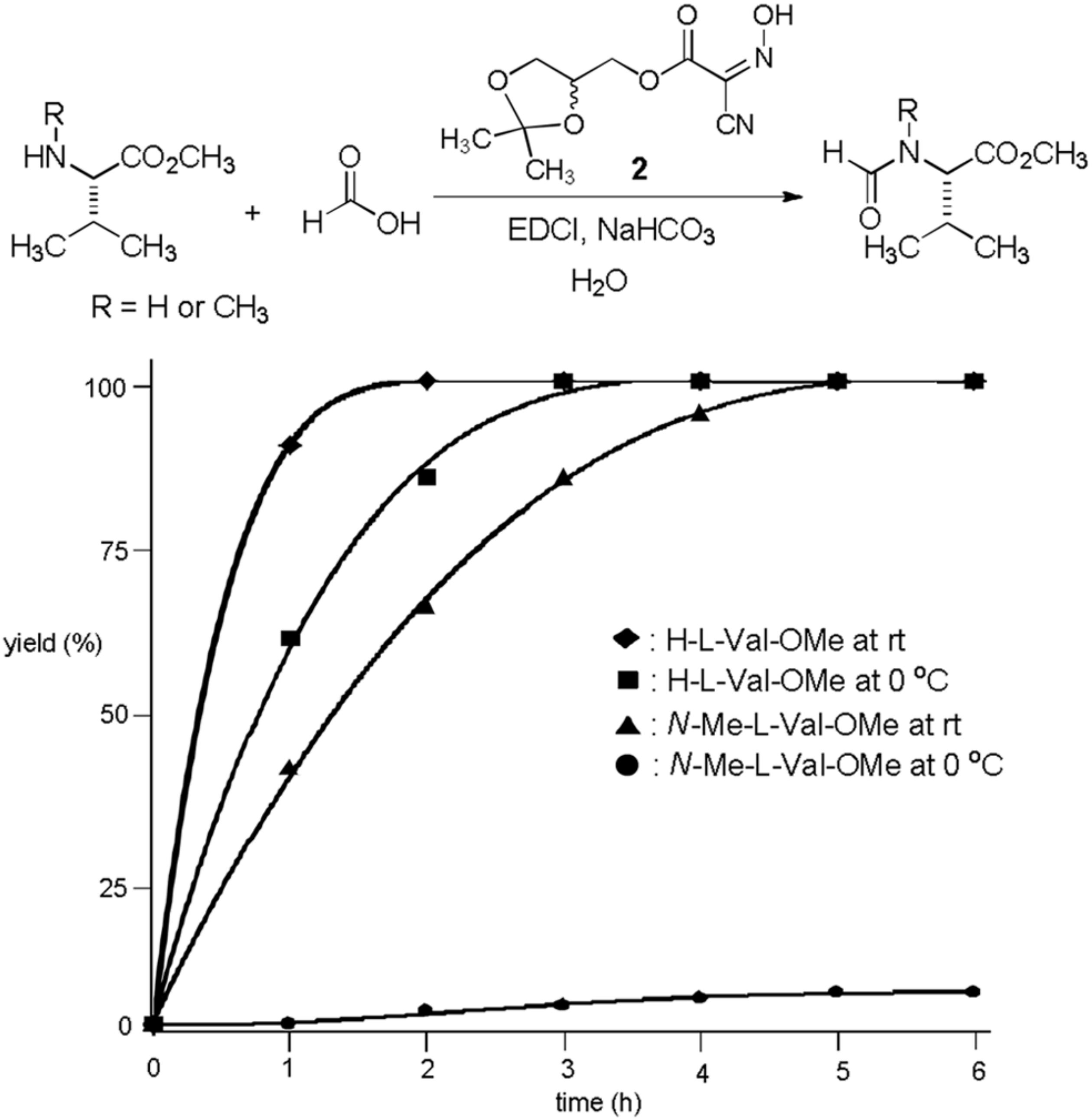
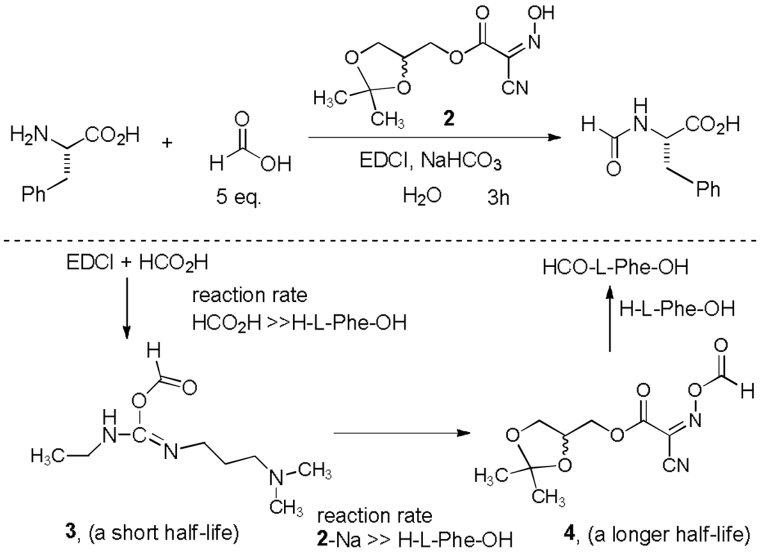


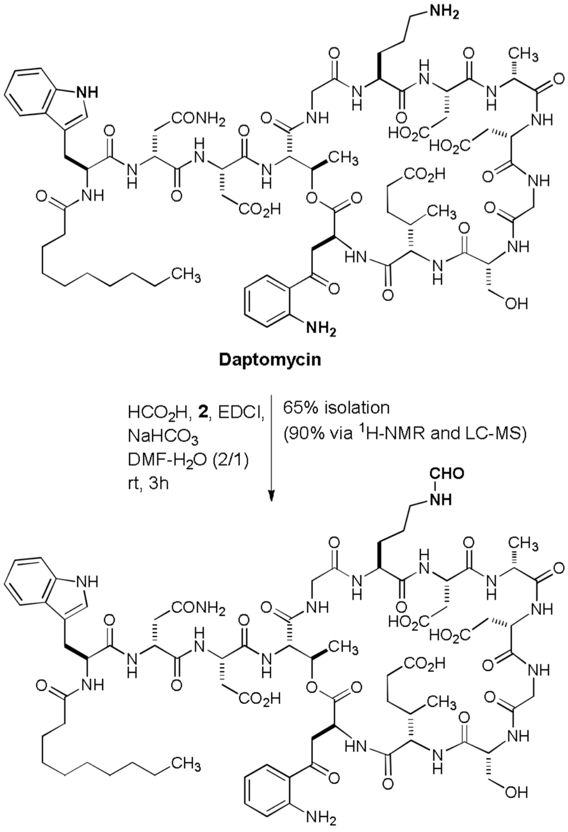
Figure 2.

The reaction kinetic curves of formylations of H-LVal-OMe and *N*-Me-L-Val-OMe in H_2O at rt and 0 °C.



Scheme 1.

Formylation of H-L-Phe-OH in water and a plausible reaction mechanism.

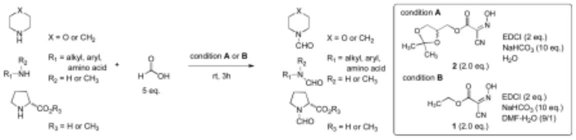


Scheme 2.

Selective formylation of daptomycin.

Table 1

N-Formylations of *primary* and *secondary* amines.[11]



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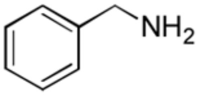
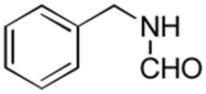
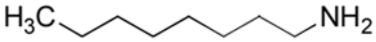
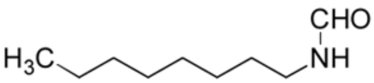
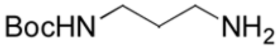
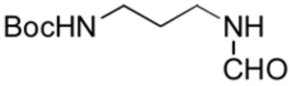
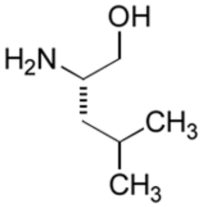
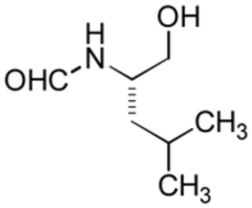
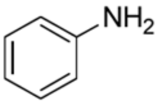
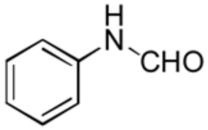
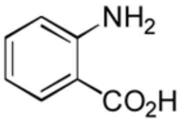
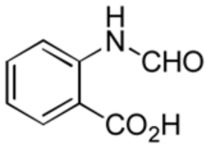
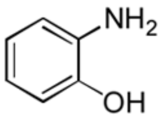
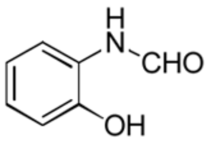
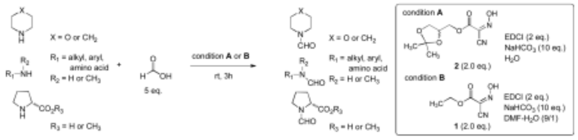
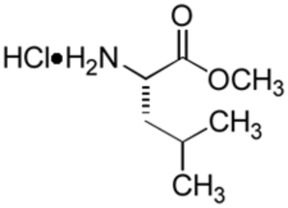
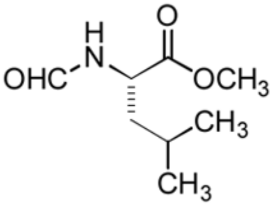
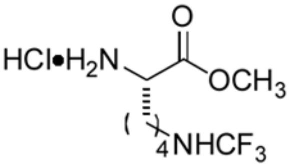
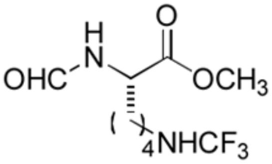
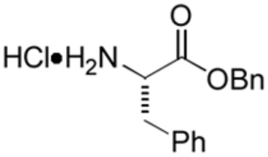
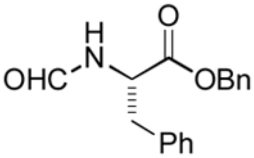
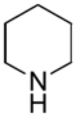
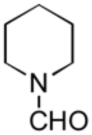
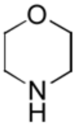
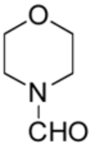
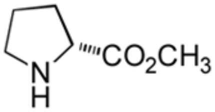
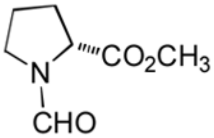
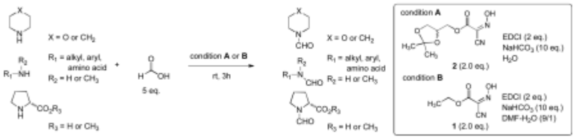
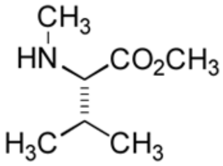
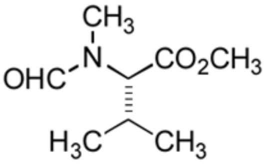
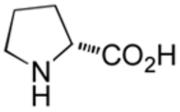
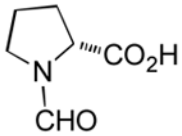
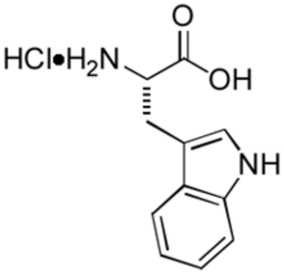
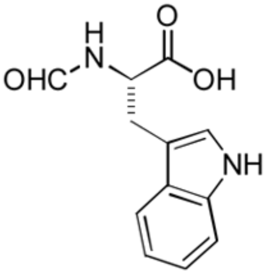
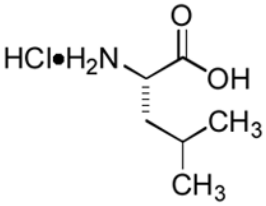
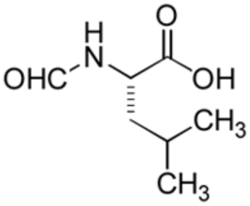
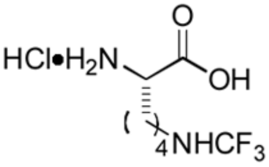
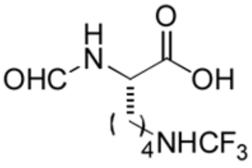
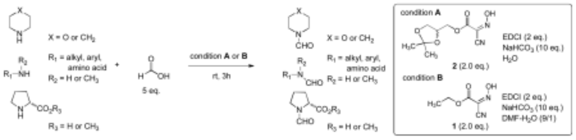
1			**B**[a]	quant.
2			**B**[a]	quant.
3			**B**[a]	quant.
4			**B**[a]	quant.
5			**B**[a]	quant.
6			**B**[a]	30
7			**B**[a]	25

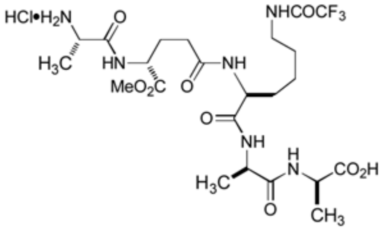
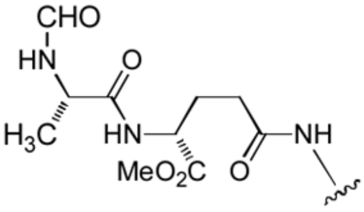
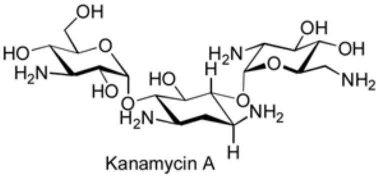
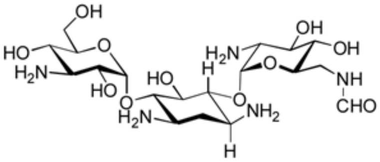
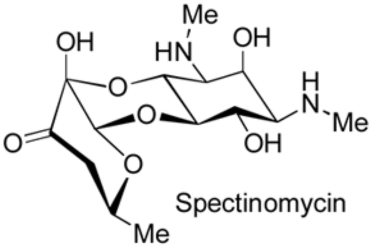
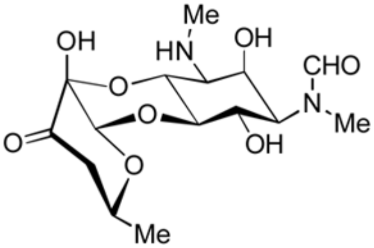


entry	starting material	product	condition	yield (%)
8			**A** or **B**	95
9			**A** or **B**	95
10			**A** or **B**	95
11			**A** or **B**	quant.
12			**A** or **B**	quant.
13			**A** or **B**	95



entry	starting material	product	condition	yield (%)
14			**A** or **B**	95
15			**A**^{b}	90
16			**A**^{b}	85
17			**A**^{b}	95
18			**A**^{b}	90



entry	starting material	product	condition	yield (%)
19			**A**	90
20	 Kanamycin A		**A**	30
21	 Spectinomycin		**A**	50

[a] The condition **A** was also effective.

[b] The same reaction under the condition **B** yielded the product in 30–60% yield.