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Regioselective Diazotransfer Reaction at the C3-Position of the 2-Desoxystreptamine Ring of Neamine Antibiotics

Andreas A. Bastian,[a] Eliza M. Warszawik,[a] Praveen Panduru,[a] Christoph Arenz,[b] and Andreas Herrmann*[a]

Aminoglycosides represent one of the largest classes of antibacterials with activity against Gram-positive and -negative bacteria. These compounds exert their antibacterial activity by binding the decoding region (A-site) of the 16S rRNA.[1] However, the increased bacterial resistance against aminoglycoside antibiotics[2] fueled activities to modify these scaffolds to obtain new active compounds.[3] In particular, the neamine moiety (1, Figure 1) of these antibiotics attracted major attention, since resistance is mainly based on the introduction of phosphate- and acetyl groups at ring I and II by the enzymes phosphotransferases (APHs) and acetyltransferases (ACCs).[4] Thus, chemical derivatizations of these compounds were carried out to overcome bacterial resistance or reduce toxicity.[5] However, these modifications require multistep synthesis[3,5a,6] due to the presence of several hydroxy and amino groups with similar reactivity. Therefore, methods were established that enabled regioselective introduction of functionalities in these antibiotics. For example, selective modifications were enabled by the application of noncovalent protective groups based on metal–chelate complexes[5e,f,g] and RNA aptamers,[5h] or employing a chemoselective Staudinger reaction.[7] However, modification at the C3-position of the 2-DOS ring remains a challenge and is so far limited to the utilization of enzymes[8] or multistep synthesis. For example, Mobashery and co-workers succeeded in the modification of this position in antibiotic 1 to reduce the electrostatic interaction of the aminoglycoside with resistance enzymes.[10] Unfortunately, this modification required multistep synthesis and thus resulted in low overall yield. To minimize the synthetic effort and enable a facile access to new antibiotic derivatives, herein we describe a one-step modification at the C3-position of ring I applicable to different structurally complex neamine antibiotics (Figure 1).

This synthetic shortcut is based on the application of the diazo-transfer reagent, imidazole-1-sulfonyl azide 7 (Scheme 1), allowing regioselective azide introduction at the least basic amino group at the 2-DOS ring in a diverse range of neamine antibiotics. This cost-efficient and scalable method does not require any protection or deprotection steps and is performed in aqueous solution by using mild conditions. The diazo-transfer reagent 7 that was applied in this work has been recently introduced as a shelf-stable, nonexplosive and water-soluble azide compound.[9] Moreover, it has proven to be a straightforward tool to convert

![Scheme 1. Diazo-transfer reaction by using imidazole-1-sulfonyl azide 7-HCl.](image-url)

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free amines into azides through an aqueous diazo-transfer reaction.

The crystalline and more stable hydrochloride salt, 7·HCl, was applied to a wide range of molecules resulting in reasonable reaction times and excellent yields. A further advantage of this reagent is that it has good functional-group tolerance and is even reactive without any catalyst, such as copper(II), nickel(II), or zinc(II). In 2011 van Hest et al. applied imidazole-1-sulfonyl azide 7·HCl to introduce a single azido group in proteins. It turned out that the N-terminus could be selectively transformed, whereas more basic amines of lysine residues were not converted at lower pH and in absence of a metal catalyst.

Inspired by this work, we tested the applicability of 7·HCl to the regioselective azide introduction to neomycin B (3), which exhibits several amino groups with pKₐ values ranging from 5.7 to 8.8 (Scheme 2). In particular, the least basic amino group at the C3-position of the 2-DOS ring (Scheme 2) seemed to be a valuable target. Thus, we explored the dependence of the reactivity of amino groups within neomycin B (3) on the pH value and the presence of copper(II) sulfate as a catalyst. All reactions were performed on a 22 mol scale of antibiotics in a mixture of corresponding antibiotic derivatives exhibiting three to six azido groups. In contrast, in the absence of copper(II) and at neutral pH values one azido group was introduced predominately reaching high conversion of neomycin B (82%) (Figure 2b and Table 1, entry 1).

In the next step, the fraction of neomycin B derivatives exhibiting one azido group was separated from the starting by HPLC. As shown in Figure 2a, the transformation of neomycin B (3) at pH 8 in the presence of copper(II) resulted in a mixture of derivatives exhibiting three to six azido groups. In contrast, in the absence of copper(II) and at neutral pH values one azido group was introduced predominately reaching high conversion of neomycin B (82%) (Figure 2b and Table 1, entry 1).

Table 1. Transformation of antibiotics by using diazo-transfer reagent 7·HCl on a µmol scale.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Antibiotic</th>
<th>Product</th>
<th>Reaction</th>
<th>Conv [%]</th>
<th>r.s. [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>8</td>
<td>18</td>
<td>82</td>
<td>90</td>
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<tr>
<td>2</td>
<td>3</td>
<td>8</td>
<td>20</td>
<td>63</td>
<td>88</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>8</td>
<td>40</td>
<td>81</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>9</td>
<td>40</td>
<td>94</td>
<td>96</td>
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<tr>
<td>5</td>
<td>2</td>
<td>10</td>
<td>40</td>
<td>46</td>
<td>96</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>11</td>
<td>40</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>12</td>
<td>40</td>
<td>96</td>
<td>97</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>13</td>
<td>40</td>
<td>97</td>
<td>98</td>
</tr>
</tbody>
</table>

[a] All reactions were performed on a 22 µmol scale of antibiotics in water adjusted to pH 6.6 with 2 mM NaOH solution and 16 equivalents of 7·HCl at room temperature. [b] Performed in 10 mM sodium phosphate buffer (pH 7) by using 8 equiv of 7·HCl. [c] Performed by using 16 equiv of 7·HCl. [d] Performed by using 8 equiv of 7·HCl. [e] Conversions of antibiotics to a mixture of corresponding antibiotic derivatives exhibiting one azido group are given and were determined by HPLC analysis. [f] Regioselectivities (r.s.) were determined by NMR spectroscopy from the mixture of mono-azido antibiotic derivatives, which were separated from the unconverted antibiotic by HPLC analysis.

Figure 2. HPLC analysis of neomycin B 3 (NeoB) after transformation by using eight equivalents of diazo-transfer reagent 7·HCl. (i–vi = number of introduced azido groups).
The selective transformation of the amino group at the C3-position can be explained by differences in the basicity of the amino groups. While five of six amines have a $pK_a$ ranging from 7.55 to 8.8, the amino group at the C3-position is the least basic one with a $pK_a$ ranging from 7.55 to 8.8. Therefore, only this amino group is accessible for the reagent 7-HCl under our identified conditions.

Moreover, to avoid the use of aqueous buffer solutions, the reaction was performed in water in the presence of sodium hydroxide to adjust an initial pH of 6.6 in the reaction mixture. Here, eight and 16 equivalents of imidazole-1-sulfonyl azide 7-HCl were applied for the transformation of antibiotic 3. It has to be noted that the pH is not changing significantly during the applied reaction times of 20 and 40 h. As shown in Table 1, using eight equivalents of 7-HCl resulted in a high conversion of neomycin B to monoazido derivatives with a regioselectivity of 90% for the modification at the C3-position (Table 1, entry 3). In contrast, employing 16 equivalents of 7-HCl gave lower regioselectivity (entry 2) and, additionally, formation of derivatives exhibiting more than one azido group were observed (see HPLC data, Figure S18 in the Supporting Information). Thus, the formation of side products gave rise to the reduced formation of monoazido derivatives (entry 2).

To test whether different antibiotics exhibiting the neamine moiety can be transformed selectively at the same position, we investigated azide introduction via 7-HCl with neamine 1, amikacin 2, paromomycin 4, ribostamycin 5, and apramycin 6 (Figure 1). Mass spectrometric analysis of the transformation of all substrates confirmed the result previously obtained for neomycin B 3 showing high reactivity of only a single amino group (see the Supporting Information, Figure S21–S25). As proven by NMR spectroscopy, all applied aminoglycosides were transformed at the C3-position of the 2-DOS ring, even when applying an excess of 16 equivalents of 7-HCl (see the Supporting Information, Figures S8–S17). All substrates were successfully transformed reaching a high regioselectivity of up to 98% for the modification of the 2-DOS ring and conversions of the antibiotics to the corresponding derivatives exhibiting one azido group between 46 and 97% were obtained (Table 1, entries 4–8). Thus, in all reactions still 3 to 54% unconverted neamine antibiotics were found.

So far, all transformations were performed on a 22 μmol scale of the antibiotics. To test the scalability of the regioselective azide introduction, we performed the diazo-transfer reaction on a mmol scale by using 16 equivalents of diazotransfer reagent 7-HCl. Moreover, the reaction was performed at a ninefold higher concentration to decrease the reaction volume. As shown in Table 2, the transformation of antibiotics 1, 3, 4, 5, and 6 resulted in very high regioselectivities and good conversions (entries 1–2 and 4–6). In contrast, the reaction of amikacin 2 resulted in low conversion, even when performed at pH 7 (Table 2, entry 3). We assume that the amino group at the C3-position of the DOS ring of aminoglycoside 2 is more basic relative to the same position of the other neamine antibiotics and, therefore, shows lower reactivity. The reason could be that the amino group at the C1-position of amikacin is acylated. This gives rise to easier protonation of the N3-position due to less repulsive forces since one positive charge in the N1-position is missing. In previous studies, it was described for neomycin B that the low $pK_a$ value of the amino group at the C3-position is due to electrostatic repulsion caused by the positive charges of the protonated amino groups at the C1 and C2-positions.[10]

The regioselective introduction of the azido group at the 2-DOS ring is highly appealing, since this functionality can be applied for further chemical diversifications through Huisgen and Staudinger reactions.[11] Thus, they can be reacted with acetylenes in a click reaction resulting in 1,2,3-triazoles and they allow the introduction of carbamates, primary amines, and amides.[12] Since both transformations,
Table 2. Transformation of antibiotics by using diazo-transfer reagent 7-HCl on a mmol scale.[a]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Antibiotic</th>
<th>Product</th>
<th>Conv.[b]</th>
<th>Yield[c]</th>
<th>r.s.[d]</th>
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<td>3</td>
<td>8</td>
<td>86</td>
<td>82</td>
<td>97</td>
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<tr>
<td>2</td>
<td>1</td>
<td>9</td>
<td>87 (63)</td>
<td>64</td>
<td>95</td>
</tr>
<tr>
<td>3[^2]</td>
<td>10</td>
<td>9</td>
<td>37 (36)</td>
<td>29</td>
<td>90</td>
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<td>77</td>
<td>95</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>12</td>
<td>79 (68)</td>
<td>71</td>
<td>93</td>
</tr>
</tbody>
</table>

[a] All reactions were performed on a 0.22 mmol scale of antibiotics in water adjusted to pH 6.6 with 2.5 mL NaOH solution and 16 equiv of 7-HCl at room temperature. [b] Performed on a 5.5 mmol scale in water by using 8 equiv of 7-HCl. [c] Isolated yields. [d] Regioselectivities (r.s.) were determined by NMR spectroscopy from the mixture of mono-7-acido antibiotic derivatives, which were separated from the unconverted antibiotic by column chromatography.

Staudinger and Huisgen reactions, can also be applied in aqueous solution, the obtained water-soluble aminoglycoside derivatives 8, 9, 10, 11, 12, and 13 might be directly transformed in a subsequent conversion to a wide range of new antibiotic derivatives without any protection and deprotection steps.

In conclusion, imidazole-1-sulfonyl azide 7 was successfully applied as a diazo-transfer reagent for regioselective azide introduction in neamine antibiotics avoiding any protective-group chemistry. We demonstrated that the reactivity of amino groups within this important class of antibiotics can be controlled by pH and addition of catalyst. Key to the single specific transformation of the amino group at the C3-position of the 2-desoxystreptamine ring is its low pKa value. The resulting regioselectivity for six different neamine antibiotics reached up to 98%. Moreover, we demonstrated the scalability of the regioselective azide introduction performing the reaction by using grams of neomycin B. Since the amino group at the C3-position of the 2-DOSt ring is a target of the resistance-causing enzyme acetyltansferases ACC(3) in Escherichia coli,[1] this facile one-step modification will certainly be useful in generating new derivatives to overcome antibacterial resistance, reduce toxicity, or study structure/activity relationships.

Experimental Section

General procedure for the regioselective azide introduction in neamine antibiotics: A solution of antibiotic sulfate salt (0.22 mmol) in water (10 mL) was combined with a solution of imidazole-1-sulfonyl azide hydrochloride (737 mg, 3.52 mmol, 16 equiv) in water (10 mL). A pH value of 6.6 of the reaction mixture was adjusted by addition of 2.5 mL NaOH solution and stirred at room temperature for 40 h before being washed twice with dichloromethane (20 mL). The aqueous solution was concentrated under reduced pressure to remove dichloromethane residues and freeze dried. The obtained crude mixture was purified by column chromatography by using a homogeneous dichloromethane/methanol/aq.

5% ammonia (from 2.5:1 to 2:3.2 v/v) mixture. After evaporation of the solvent the residue was resolved in water (3 mL) and traces of silica were removed by filtration through 0.45 μm syringe filters. Lypoophilization yielded the 3C-acido aminoglycoside antibiotic.

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Keywords: antibiotics · diazo-transfer · drug design · modification · regioselectivity


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