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Structural elucidation of biologically active neomycin *N*-octyl derivatives in a regioisomeric mixture by means of liquid chromatography/ion trap time-of-flight mass spectrometry

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Structural elucidation of six regioisomers of mono-*N*-octyl derivatized neomycin is achieved using MS^n (up to n = 4) on an ion trap time-of-flight (IT-TOF) instrument equipped with electrospray ionization. The mixture of six derivatized neomycin analogues was generated by reductive amination in a shotgun synthetic approach. In parallel to the liquid chromatography/mass spectrometry (LC/MS) detection, the antibacterial activity of the neomycin regioisomers was tested by post-column addition of buffer and bacterial inocula, subsequent microfractionation of the resulting mixture, incubation, and finally a chemiluminescence-based bioactivity measurement based on the production of bacterial ATP. The MS-based high-resolution screening approach described can be applied in medicinal chemistry to help in designing and producing new antibiotic substances, which is particularly challenging due to the high functionality of most antibiotic substances, therefore requiring advanced (hyphenated) separation and detection techniques for compound mixtures. Copyright © 2010 John Wiley & Sons, Ltd.

In the past few years, we have been developing hyphenated and mass spectrometry (MS)-based analytical technologies enabling high-resolution biological activity screening of (complex) mixtures and simultaneous identification of active sample constituents.[1–3] Such a hyphenated continuous-flow system usually consists of an initial chromatographic separation step, followed by the on-line addition of all necessary biochemical reagents. MS can then be used to monitor either the column effluent (analyte detection) or additionally the biochemical reaction. One of the disadvantages of such a continuous-flow approach is that it is only applicable to relatively fast biological reactions. Additionally, reaction constituents may hinder efficient MS detection. In order to keep the benefits of high-resolution screening assays, but enabling longer reaction times in the bioassay, we have recently described a microfractionation system.[4] Such an approach has the additional advantage that the bioactivity screening and the mass spectral identification step are essentially decoupled (parallel assay), which allows the use of chemicals in the bioassay which are not at all amenable to MS detection. This is especially true if the bioactivity screening would involve bacteria in order to screen for new antibacterial agents. The screening for antibacterial substances is usually carried out by incubating a small number of colony-forming units (CFU) in the presence or absence of test substances in a suitable broth medium.[5] After incubating the bacterial inocula under suitable conditions for 18 h or longer, the growth is monitored visually, or by the use of a UV spectrophotometer. The concentration of a substance which just does not lead to a detectable bacterial growth is the so-called minimal inhibitory concentration, or MIC. Another more sensitive readout possibility for MIC determinations is the detection of ATP, which is formed by metabolically active bacteria and is directly proportional to the number of bacteria found under standardized conditions.[6]

In the ongoing struggle to find new antibiotics, complex mixtures like natural extracts are still the most prominent source for antibiotic substances[7,8] or lead structures which are synthetically modified.[9] The high numbers of functional groups and stereocenters of most antibiotics make it very difficult to synthetically address selected groups of an antibiotic scaffold without rebuilding the backbone with modified building blocks. These and other difficulties in producing novel antibiotic derivatives make it necessary to find new strategies where separation sciences, MS, microbiology, and medicinal chemistry are combined in a single platform to overcome bottlenecks of today's drug discovery processes.[10]

The model compound used in this study is the aminoglycoside antibiotic neomycin, which is an RNA targeting substance.[11] Recently, it has been shown that neomycin lipid

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conjugates can show potent antibacterial activity.[12] Moreover, a *N*-per-arginylated neomycin derivative was shown to be an antagonist of HIV Tat protein.[13] Both recent developments inspired us to study the reductive amination[14,15] of neomycin in a one-pot shotgun approach towards *N*-alkylated derivatives and to provide a new strategy to elucidate the bioactivity and structures of these closely related substances generated in this way.

The general setup of the fully integrated high-resolution screening system is described here. It comprises of liquid chromatography for the separation of complex (reaction) mixtures, a split towards MS detection and identification on one hand and the microfractionation system towards bioactivity assessment on the other. The bioactivity of the various mono-*N*-alkylated neomycin derivatives is compared. The structural elucidation of all neomycin derivatives was accomplished by liquid chromatography/ion trap time-of-flight (LC/IT-TOF) MS^n experiments and is discussed in detail.

EXPERIMENTAL

Chemicals and bacteria

Bactiter Glo reagent was purchased from Promega (Leiden, The Netherlands). Piperacillin dry substance (Ratiopharm, Ulm, Germany) was from a local pharmacy. All other chemicals were purchased from Sigma Aldrich (Schnelldorf, Germany). We used *E. coli* BL21 (DE3) as model organism maintained in Müller Hinton II broth (MHII) (33 g/L).

Generation of *N*-alkylated neomycins

Stock solutions of 10 mM neomycin trisulfate (minimum 85% neomycin B according to the supplier) in 20 mM phosphate buffer (pH 6.5), 125 mM octanal in MeOH, and 200 mM $NaCNBH_3$ in 1:1 phosphate buffer/MeOH were prepared. The octanal stock solution was diluted 1:10 with phosphate buffer resulting in the working solution. Volumes of 200 μL of each solution (1.25 equiv octanal) were mixed and heated to 60°C for 2.5 h. The resulting solution was subsequently diluted (1:20) with LC solvent A and used without any further cleaning for bioactivity screening.

Instrumentation and procedures

The general setup of the fully integrated MS-based high-resolution screening system is shown in Fig. 1. The system consists of four units: an LC separation system, an on-line MS detection device, a microfractionator, and a plate reader, where the bioactivity measurement after incubation is performed.

LC separation

A LC20 AB quaternary pump (Shimadzu, s'Hertogenbosch, The Netherlands) was used to deliver a mixture of water/acetonitrile (ACN) at 60 μL/min (solvent A: water/ACN 95:5, 0.1% acetic acid, 0.02% trifluoroacetic acid (TFA), and solvent B: ACN/water 95:5, 0.1% acetic acid). The autosampler was a Shimadzu SIL 20 A. For all experiments, an injection volume of 5 μL was used. The column used was an Ultra C_{18} (50 × 1 mm, 3 μm; Restek GmbH, Bad Homburg, Germany). The column oven was held at 40°C. A post-column splitter (T-split, Valco, Schenkon, Switzerland) was used to split the LC flow in a 1:2 ratio (MS:microfractionator), that is 20 μL/min to the MS and 40 μL/min to the bioassay.

On-line MS detection

A Shimadzu LC20 AD was used to deliver the MS make up flow (ACN/water 60:40, 0.1% acetic acid, 0.02% TFA) at a flow rate of 80 μL/min leading to a total flow towards the mass spectrometer of 100 μL/min. The mass spectrometer used in the on-line bioactivity assay was a Waters Micromass Ultima Q-TOF (Waters, Milford, MA, USA). The mass spectrometer was operated with electrospray ionization in positive-ion mode during all experiments. The capillary voltage was 2.5 kV, source temperature 120°C, desolvation temperature 350°C, and the cone voltage was 35 V. Nitrogen (99.9990%) was used at flow rates of 50 L/h as cone gas and 400 L/h for desolvation. MS data was acquired in the range *m/z* 220–1250 with a spectrum acquisition rate of 1 spectrum/s.

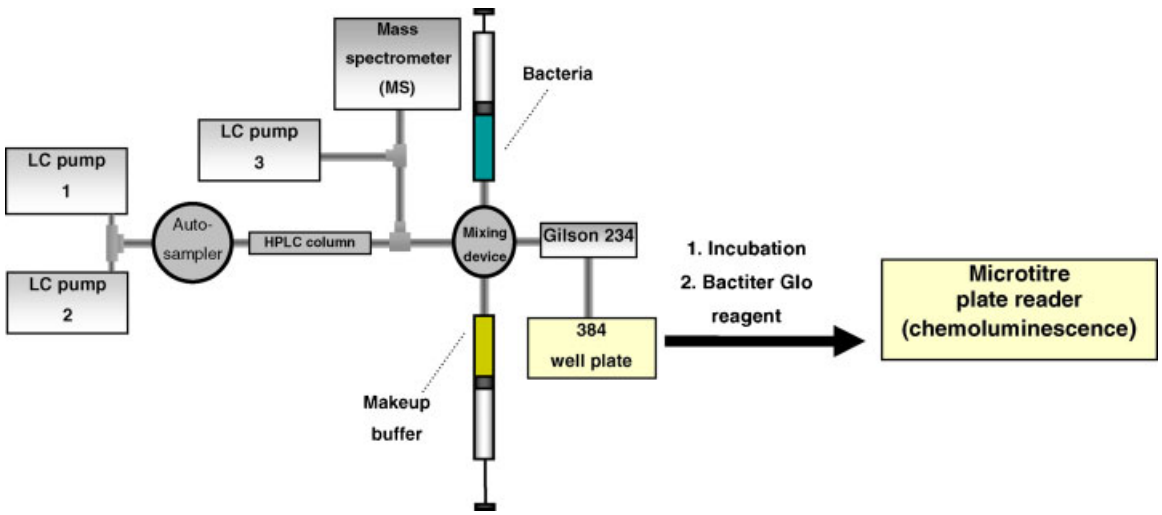

Figure 1. Schematic overview of the fully integrated MS-based high-resolution screening system. For details, refer to the text.

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Microfractionation

All experiments were carried out with the Gram-negative bacterium *E. coli* (strain BL21 (DE3)) as model organism. Bacterial inoculum (1:500 dilution of an overnight culture in MHII medium, 66 g/L, about 1.1×10^6 cells/mL, according to a 0.5 Mc-Farland standard[5]) and make-up buffer solution (Tris 20 mM, pH 10.1) were pumped by a model 22 two-channel syringe pump (Harvard Apparatus, South Natick, MA, USA) at a flow rate of 40 μL/min. A model 234 autosampler (Gilson, Middleton, USA) served as fractionator. It was programmed to collect fractions of 6 s, resulting in 12 μL fractions. The software controlling the Gilson 234 was home-made. The needle of the 'autosampler' was replaced by a 150 μm i.d. deactivated fused-silica capillary, of which the last cm of the polyimide coating was removed and the residual quartz glass was silylated using dimethyldichlorosilane in toluene (5%) for 3 min. The 384 low-volume well plates (solid, white) were from Brand (Wertheim, Germany).

Incubation and bioassay

The incubations of the LC fractions with the *E. coli* bacteria were carried out at 36°C for 18 ± 2 h under a humidified atmosphere. After incubation, 12 μL of the Bactiter Glo reagent were pipetted into each well using an eight-channel multipipette. After addition of the Glo reagent, plates were read at the Victor^3 plate reader (Perkin Elmer, Waltham, MA, USA) after an additional incubation time of 5 min. The plate reader counted chemiluminescence for 1 s per well with the aperture set to 'normal'. The readout was based on the measurement of ATP produced by bacterial growth.[6] The levels of ATP are directly related to the number of metabolically active bacteria within the incubation broth.

For the determination of minimal inhibitory concentrations (MIC), the system was operated under isocratic conditions, taking care that the inhibitor substances eluted without retention: 40% eluent B was used for piperacillin and 25% B for neomycin. All injections were carried out in duplicate.

MS^n structural elucidation

The MS^n experiments for structural elucidation were carried out using a Shimadzu IT-TOF instrument equipped with an ESI source operated in positive-ion mode. The curved desolvation line and the heating block were set to 200°C, the interface voltage was set to 5 kV, while a voltage of 1.7 kV was applied for the detector. Nitrogen (99.9990%) was used as a nebulizing gas at a flow of 1.5 L/min and a drying gas flow of 10 L/min. For the fragmentation experiments, argon (99.9995%) was used as collision gas. In the full spectrum mode, data was acquired from *m/z* 100–800, with an ion accumulation time of 10 ms. MS^2 experiments were performed on the ion with *m/z* 364.226 with an ion isolation time of 20 ms and a frequency of 45 kHz. Both collision energy and gas were set to 50%. With an isolation time of 20 ms, MS^3 experiments were performed on the ion with *m/z* 567.360, using 100% collision energy and gas and a frequency of 45 kHz. MS^4 settings were 30 ms isolation time, 100% collision energy and gas, frequencies of 52 kHz and/or 83 kHz on the ions with *m/z* 273.217 and 275.232.

RESULTS AND DISCUSSION

Biological screening

The biological screening procedure (Fig. 1) is based on the continuous-flow on-line addition of all necessary reagents (*E. coli* bacterial inoculum and TRIS buffer for pH correction) after LC separation, subsequent microfractionation into a 384-well low-volume microtitre plate (12 μL fractions), 18-h incubation, and a chemiluminescence-based bioactivity readout based on the measurement of ATP produced by bacterial growth using the Bactiter Glo reagent.[6] The reagent extracts intracellular ATP and determines its amount with a recombinant firefly luciferase, thereby producing light. The levels of ATP are directly related to the number of metabolically active bacteria within the incubation broth. In assessing bioactivity of antibacterial agents, this means that a high baseline in the bioactivity trace is observed when no antibacterial agent is present and the bacterial growth can occur without inhibition, whereas negative peaks indicate the presence of bioactive compounds in the effluent stream. To evaluate the effectiveness of different substances, it is necessary to compare their peak heights obtained in the bioassay, as the peak height is directly proportional to the caused effect and, unlike the peak area, does not increase anymore when a 100% response is reached. As only two-thirds of the LC effluent is used in the bioassay, simultaneous MS detection and identification of the bioactive substances in the mixture using the other third of the LC effluent can be achieved using a QTOF instrument.

Prior to its application to the bioactivity screening of *N*-alkylated neomycin derivatives, the screening platform was characterized using piperacillin and neomycin as model compounds. Some of the assay characteristics of the described screening platform are shown in Table 1. The obtained MIC values proved to be comparable to published values. Usually, MIC values are determined by performing serial 1:1 dilutions of the substance under investigation.[16] In many cases, the readout is still performed visually:[17] the MIC

Table 1. Assay characteristics

Substance	Found MIC[ab]	Ref.: Strain/MIC[ab]
Piperacillin	0.87 μg/mL	*E. coli* K-12 C600/1.56 μg/mL[19]
Neomycin	1.04 μg/mL	*E. coli* ATTC27853/4 μg/mL[12]
Substance	Intra-day repeatability (n = 3), conc.[a]; RSD[cd]	Inter-day repeatability (n = 3), conc.[a], RSD[cd]
Neomycin	50 μg/mL; 17.8 (%)	25 μg/mL; 16.2 (%)
Piperacillin	45 μg/mL; 11.7 (%)	30 μg/mL; 2.3 (%)

[a] The concentrations refer to the injected solutions.
[b] All MIC values were corrected for the dilution factor, as described elsewhere.[4]
[c] RSD: relative standard deviation.
[d] The peak 'heights' obtained in the biological assay were compared.

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value is the concentration of tested substance at which no bacterial growth can be visually detected. Hence, MIC values differing by a factor of 2–4 are not unusual, moreover because these values are strongly dependent on incubation conditions and used bacterial strain. Figure 2 shows a comparison of the bioactivity data and the mass spectrometric data obtained during the MIC determination of neomycin. It can be seen that no bacterial growth was detected when a neomycin concentration of 10 μg/mL was injected. This, of course, is also true for the injection of a concentration of 25 μg/mL, as the maximum biological effect is already reached. At the same time, it can be seen that at a higher injected concentration the observed signals in the biological trace become broader, due to band broadening effects. Therefore, the peak height is corresponding to the assay window (0–100% inhibition), as the peak area would steadily increase with higher concentrations although a maximal biological effect was already reached. This is why we used the peak heights of the biological data and not the peak areas for further comparisons. Acceptable intra-day and inter-day repeatability was obtained with relative standard deviation (RSD) below 20%. This indicated that all parameters proved to be acceptable for a screening technology. The effect of the organic modifier acetonitrile was studied by performing a water injection and applying a gradient from 0 to 90% eluent B in 10 min with a hold time of 3 min. Under these conditions, no changes in the biological signal (that is in the bacterial growth) were observed. Nevertheless, the maximum amount of eluent B was restricted to 80%, as broth components started to precipitate at about 90% of eluent B, as visually determined.

Generation of *N*-alkylated neomycin derivatives

The one-pot shotgun approach applied to generate *N*-alkylated neomycin derivatives by reductive amination[14,15] results in a complex mixture of basically three groups of products, that is unreacted neomycin, mono-*N*-alkylated neomycin derivatives, and products from multiple *N*-alkylation. In the present study, we focused on the mono-*N*-alkylated neomycin derivatives, which are the major reaction products under the reaction conditions used (1.25 equiv. of aldehyde, *n*-octanal in our case). The other two groups of products are readily separated from the mono-*N*-alkylated neomycin derivatives by LC.

In principle, neomycin gives six degrees of freedom for a mono-*N*-alkylation reaction. Figure 3 exemplifies one of the six *N*-octyl neomycin derivatives, generated by reductive amination using *n*-octanal, representing an *n*-octyl chain on one of the six nitrogen atoms, that is the amino groups on the amino sugar rings 1, 2, and 4. Although the pK_a values of the nitrogen atoms are quite different,[18] which may influence the relative conversion efficiency, all six possible neomycin-*N*-octyl derivatives were detected.

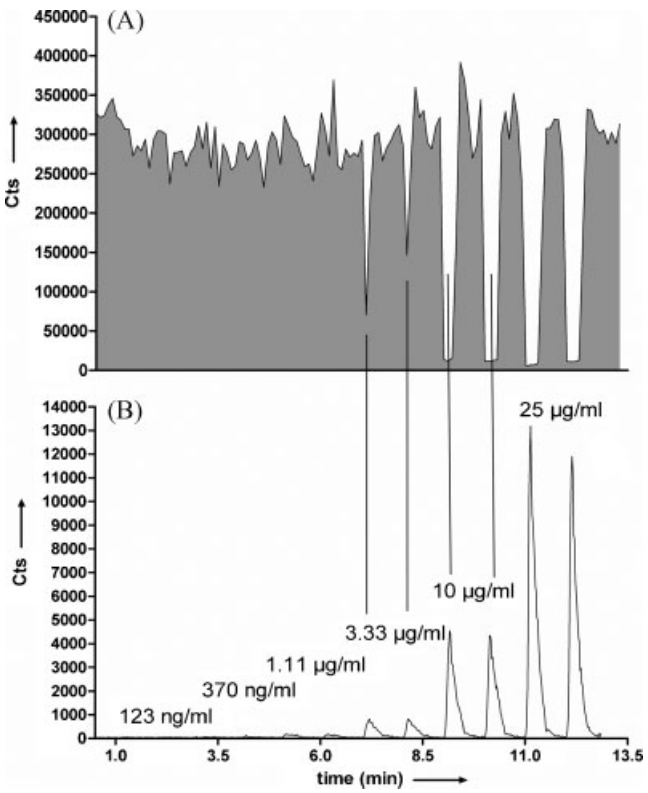

Figure 2. MIC determination of neomycin: (A) biological response, (B) EIC, *m/z* 615.5. All injections were performed in duplicate; the given concentrations refer to the injected solutions.

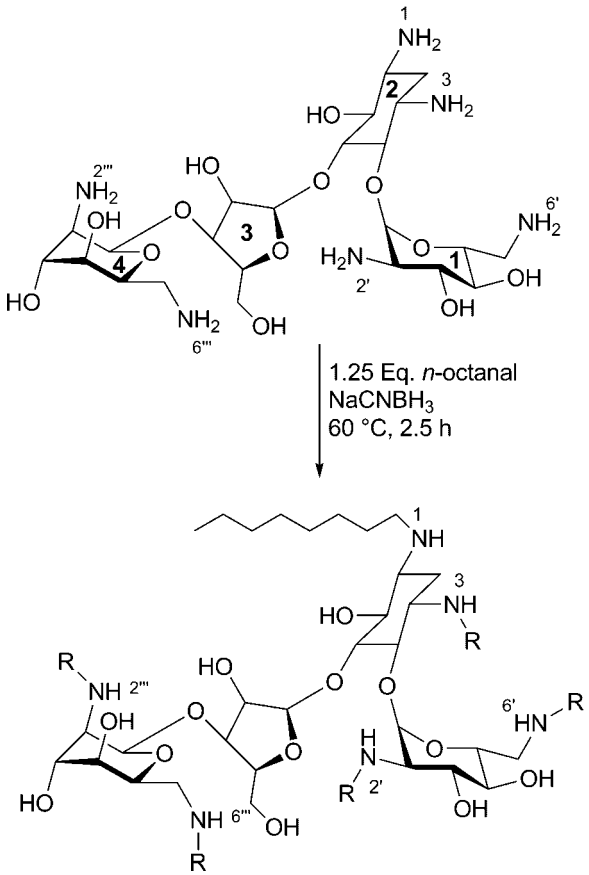

Figure 3. Reductive amination of neomycin. The alkylated derivatives have a single *n*-octyl chain on one of the six nitrogen atoms, as exemplified for N-1. Ring numbers (in the ring) and nitrogen numbers are specified. R = H or *n*-octyl.

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Bioactivity screening of an *N*-octyl neomycin shotgun mixture

The produced regioisomeric mixture of *N*-octyl neomycin derivatives was 1:20 diluted and analyzed with the high-resolution screening platform developed. A typical chromatogram obtained is shown in Fig. 4. The top chromatogram was reconstructed from the plate reader data of the individual 12 μL fractions after microfractionation and incubation, whereas the bottom chromatogram is an extracted ion chromatogram for *m/z* 727.5 and 364.3, being the singly and doubly charged ions of the *N*-octyl neomycin derivatives. It can be seen that the six generated regioisomers were successfully separated and simultaneously screened for bioactivity. At the present concentration, the substances 4, 5 and 6 showed full growth inhibition, whereas the substances 1, 2 and 3 did not exhibit any growth reduction. Further experiments with other dilution factors of the reaction mixture showed that especially compounds 4 and 6 show significant growth inhibition. A more quantitative assessment of the bioactivity of the various mono-*N*-octyl neomycin derivatives is hampered by the fact that the various analogues are generated in different ratios and (relative) quantification is difficult due to the lack of a chromophore, which would enable UV detection. Given the great influence small structural changes may have on the ionization efficiency in ESI, the observed MS response cannot be applied for relative quantification. The present discussion is primarily focused on a proof-of-principle of the MS-based high-resolution screening platform and on the structural elucidation of the 6 mono-*N*-octyl neomycin derivatives.

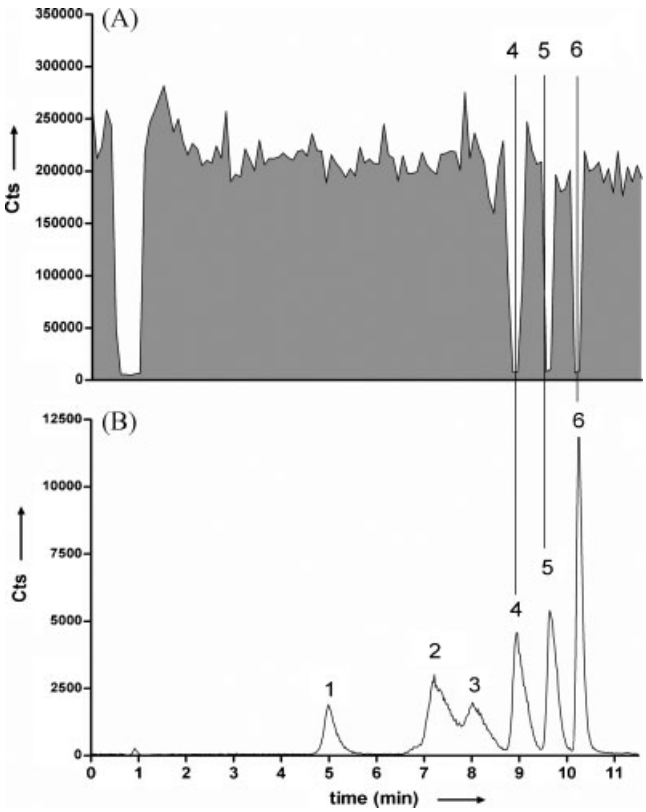

Figure 4. Separation with MS detection and simultaneous bioactivity screening of a 1:20 diluted neomycin shotgun reaction mixture: (A) the bioactivity trace and (B) the extracted-ion chromatogram for the ions with *m/z* 727.5 + 364.3.

High-resolution MS^n identification of the formed derivatives

The different neomycin derivatives were identified by high-resolution MS^n experiments using an IT-TOF hybrid instrument coupled to LC. The full MS spectra of all six mono-*N*-octyl neomycin derivatives described are identical, showing doubly and triply charged ions, $[M+2H]^{2+}$ and $[M+3H]^{3+}$ (see Fig. 5(A)). Note: under the described conditions in the IT-TOF source, mainly doubly and triply charged ions were observed, whereas in the Z-spray source on the QTOF, mainly singly and doubly charged ions were observed. For all derivatives, the doubly protonated molecules $[M+2H]^{2+}$ gave the most intense signals, and these were therefore subjected to fragmentation studies.

Upon fragmentation, all doubly charged derivative ions showed the loss of a terminal amino sugar ion (*m/z* 161.091) leading to the complementary singly charged fragment ion with *m/z* 567.362 (see Fig. 5(B)). When the octyl modification is on ring 2 (cf. Fig. 3), the fragment ion with *m/z* 567.362 can be generated by the loss of either of the isomeric amino sugar rings 1 or 4. When alkylation occurs at ring 1, the fragment ion can only be generated by the loss of ring 4, and *vice versa*.

MS^3 experiments lead to the first observable differences between the spectra. Four out of six *N*-octyl neomycin derivatives (peaks 1, 3, 4 and 6 in Fig. 4) clearly showed a fragment ion with *m/z* 273.217 (Fig. 6), while the MS^3 experiments on the remaining two derivatives (peaks 2 and 5) generated the fragment ion with *m/z* 275.233.

The fragment with *m/z* 275.233 corresponds to the loss of rings 1 and 3, indicating that the site of alkylation in peaks 2 and 5 is at ring 2. MS^4 experiments on the fragment ion with *m/z* 275.233 resulted in identical spectra for both derivatives, as is expected since alkylation on either of the nitrogen atoms

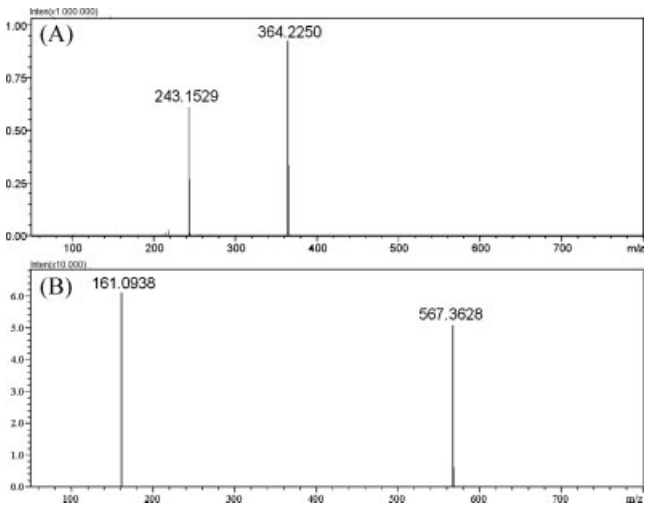

Figure 5. (A) MS^1 and (B) MS^2 spectrum on the ion with *m/z* 364 of peak 4. For details, refer to the text.

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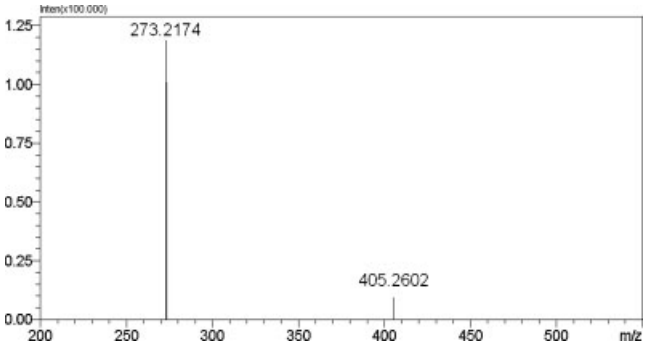

Figure 6. MS^3 spectrum on *m/z* 567.362 of peak 4.

1 and 3 in ring 2 results in identical structures in the symmetric fragment ion obtained after the loss of all three unmodified rings. Therefore, no further discrimination between these two compounds is possible.

Figure 7 shows a fragmentation scheme for the different observed ring losses, summarizing the fragmentations described here. Next to the fragment ion with *m/z* 273.217, the MS^3 spectra of peaks 3 and 4 contained a fragment ion with *m/z* 405.260 originating from the loss of ring 2, thus indicating that the site of alkylation in peaks 3 and 4 is at ring 4 (see Fig. 6). The presence of a fragment ion with *m/z* 435.317 (loss of ring 3) in the MS^3 spectra of peaks 1 and 6 confirms that the alkylation is on ring 1.

Subsequent MS^4 experiments on the ion with *m/z* 273.217 resulted in the formation of the fragment with *m/z* 226.183 for two out of four derivatives (peaks 1 and 3). This fragment is consistent with the loss of H_2O and $H_2C{=}NH$, that is due to cleavage of the C–C bond at the alkylated ring. This fragment is crucial for the identification, since it is only likely to occur from the isomer with a free $-CH_2-NH_2$ group, leaving the ring structure intact. Additionally, a fragment

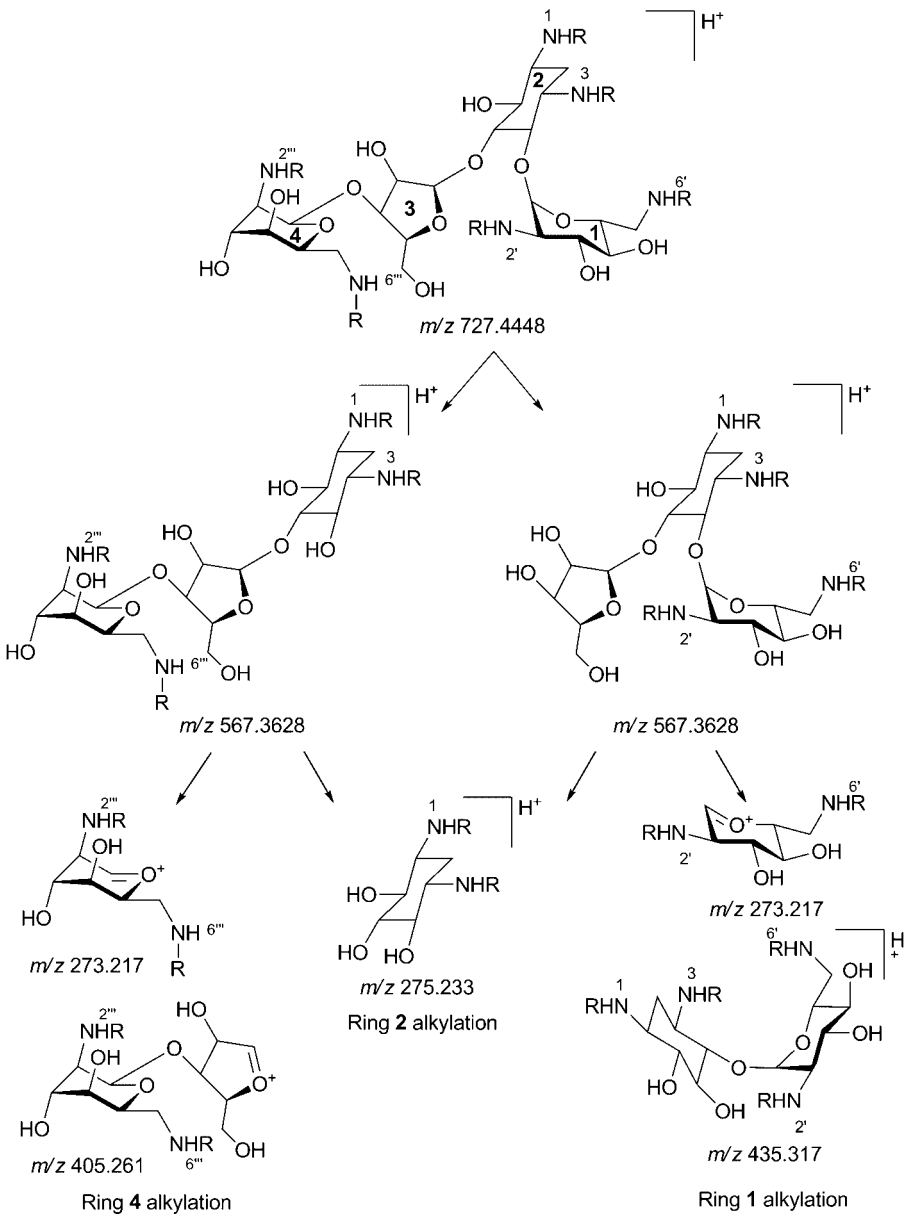

Figure 7. Fragmentation scheme of mono-*N*-alkyl neomycin derivatives in MS^n experiments, showing the different ring losses and the calculated *m/z* values of the resulting fragment ions. R = *n*-octyl or H.

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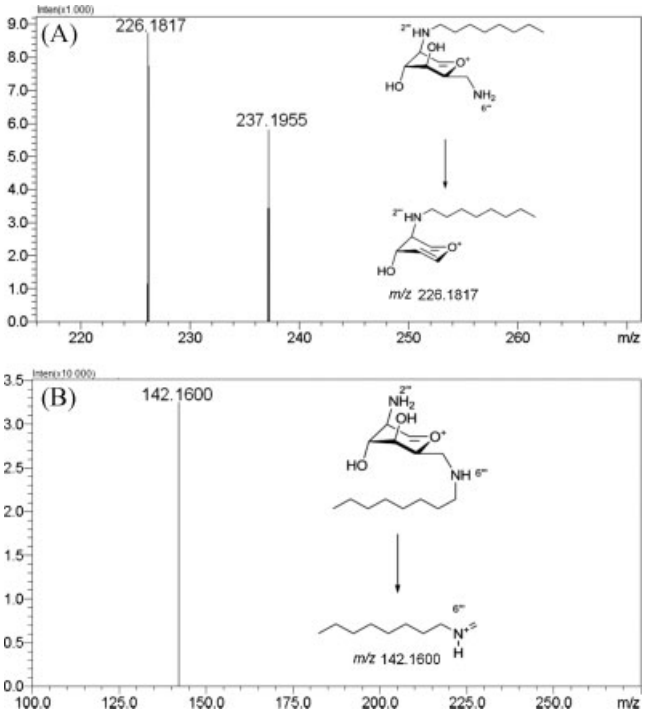

Figure 8. MS^4 spectra on *m/z* 273.217 of peak 3 (A) and 4 (B), with alkylation on either the methylamino group or the 'ring' amino group.

with *m*/z 237.1955 was produced which corresponds to a loss of two water molecules. Figure 8(A) shows the MS^4 spectrum and exemplifies the described MS^4 fragment ion for substance 3. Other MS^4 fragment ions were observed for peaks 4 and 6, as is for instance demonstrated for peak 4 in Fig. 8(B).

The fragmentation of the mono-*N*-alkyl neomycin derivatives in MS^3 and MS^4 allowed the identification of the derivatives, except discrimination between peaks 2 and 5. The results are summarized in Table 2. All reported *m*/z values of fragment ions in MS^3 experiments were within 3 ppm error of the exact mass, while all relative mass errors of fragment ions in MS^4 experiments were within 9 ppm.

CONCLUSIONS

A fully integrated high-resolution screening platform to assess growth inhibition by antibacterial substances was described. The described biochemical assay gave acceptable characteristics to be used as a screening approach for antibacterial activity. The obtained MIC values were comparable to the literature values. The ability to screen for bioactive substances in a complex mixture was shown for the generated shotgun mixture of the *N*-octyl neomycin derivatives. A core feature of the screening platform truly is MS, as it enables sensitive detection and (possibly) identification of active substances. In the presented case, we showed that high-resolution MS^n experiments on an IT-TOF instrument can lead to a full structural assignment of bioactive compounds. The assignment of the alkylated ring in the *N*-octyl neomycin derivatives was accomplished by investigating the subsequent cleavages of the glycosidic bonds. Ultimately, we were able to specify the *N*-alkylation site in rings 1 and 4 by the fact that the methylene-amino derivatives showed a characteristic fragmentation in the MS^4 experiments. The alkylation position at ring 2 was not distinguishable due to the symmetry of the MS^3 fragments. The presented screening platform was designed for the fully integrated screening of closely related substances such as the *N*-octyl neomycin derivatives, for example in structure activity studies of modified core structures. It has to be noted

Table 2. Identification of neomycin derivatives

Derivative (peak no. according to Fig. 4)	Alkylated ring number	Alkylated nitrogen atom	Characteristic fragments[a] [*m/z*] in MS^3 (#) and MS^4 (*)	Calculated *m/z*	Chemical formula
1	1	2′	#435.317	435.318	$C_{20}H_{43}N_4O_6^+$
			#273.217	273.217	$C_{14}H_{29}N_2O_3^+$
			*226.183	226.181	$C_{13}H_{24}NO_2^+$
2	2	1 or 3	#407.276	407.276	$C_{19}H_{39}N_2O_7^+$
			#275.233	275.234	$C_{14}H_{31}N_2O_3^+$
			*258.208	258.207	$C_{14}H_{28}NO_3^+$
3	4	2‴	#405.260	405.260	$C_{19}H_{37}N_2O_7^+$
			#273.217	273.217	$C_{14}H_{29}N_2O_3^+$
			*237.196	237.196	$C_{14}H_{25}N_2O^+$
			*226.180	226.181	$C_{13}H_{24}NO_3^+$
4	4	6‴	#405.260	405.260	$C_{19}H_{37}N_2O_7^+$
			#273.217	273.217	$C_{14}H_{29}N_2O_3^+$
			*142.160	142.160	$C_9H_{20}N^+$
5	2	1 or 3	#407.276	407.276	$C_{19}H_{39}N_2O_7^+$
			#275.233	275.234	$C_{14}H_{31}N_2O_3^+$
			*258.206	258.207	$C_{14}H_{28}NO_3^+$
6	1	6′	#435.317	435.317	$C_{20}H_{43}N_4O_6^+$
			#273.217	273.217	$C_{14}H_{29}N_2O_3^+$
			*142.160	142.159	$C_9H_{20}N^+$

[a] found *m*/z, the relative error [ppm] for all MS^3 measurements was below 3 ppm and for all MS^4 measurements below 9 ppm.

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that a full structural elucidation by means of MS^n experiments is much more difficult, if not impossible, when screening for totally unknown substances, for example in natural extracts or fungal broths tested for their antibacterial activity. Nevertheless, the presented platform might be helpful even in these cases for a first tracking of bioactive components, followed by isolation and full structural elucidation using NMR techniques.

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