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Unexpected α,β-unsaturated products of reductive amination of the macrolide antibiotic josamycin

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ABSTRACT

Reductive amination of the macrolide antibiotic josamycin with alkyl amines, using three different reducing agents: $NaBH_3CN$, $NaBH_4$ and $NaBH(OAc)_3$, yields surprisingly different major products which are identified as either Lewis complexes, aminoalkyl derivatives or α,β-unsaturated derivatives of josamycin by ^1H and ^{13}C NMR, FT-IR and ESI MS methods.

Josamycin **1** (leucomycin A_3) (Fig. 1) is a macrolide belonging to the leucomycin group of antibiotics.[1] Macrolides are often classified according to the size of the macrolide ring;[2] josamycin belongs to the 16-membered class of macrolides and is substituted with a 4-*O*-isovalerylmycarosylmycaminose sugar moiety. Josamycin is produced by *Streptomyces narbonensis var. josamyceticus* and was discovered by Umezawa et al. in 1967.[3] This antibiotic, similar to other leucomycins, has a broad spectrum of antimicrobial activity against Gram-positive and erythromycin-resistant bacteria, for example, *Streptococcus pyogenes* and *Streptococcus pneumoniae* at low concentration levels.[4–7] Previously, it was reported that the immunomodulatory properties of josamycin may have clinical relevance to modulation of the immune response in transplant patients and in patients with inflammatory diseases.[8] As safe and well-tolerated antibiotics, macrolides such as josamycin play an important role in the treatment of respiratory tract infections[6,9] and they are often used in combination therapy for the treatment of some forms of cancer.[10,11] The mechanism of macrolide activity is based on the inhibition of bacterial protein biosynthesis by binding reversibly to the 50S subunit of the bacterial ribosome which leads to the inhibition of translocation of peptidyl tRNA.[12]

Antimicrobial resistance among respiratory tract pathogens,[13–15] and in some cases allergy[16,17] to macrolides is a growing problem and hence there is continuous need for the synthesis of new and effective compounds of this type. To date, josamycin and other leucomycins have been most frequently modified at the C-3″ carbon atom[18] of the isovalerylmycarose moiety, and the C-9[19,20] and C-3[21] carbon atoms within the aglycone moiety which lead to ether and ester derivatives of this macrolide. Josamycin forms acetals in acidic methanol, ethanol, propanol and butanol solutions,[22] whereas in aqueous acidic media, allylic rearrangement within the conjugated dienol system leads to the formation of isojosamycin[23] possessing a hydroxy group at C-13. Under alkaline treatment josamycin is converted into its bicyclo lactone derivatives as a result of an intramolecular substitution reaction between the carbanion α to the aldehyde group and the acetate group at C-3, as reported by Omura et al.[24] Recently the conversion of 16-membered leucomycins into 14-membered aza-erythromycins[25] by metathesis, and into their bicyclic derivatives[26] by Diels–Alder reactions were described. Treatment of josamycin with $NaBH_3CN/CH_3COO^-NH_4^+$ yields the dimeric aza-derivatives, whereas the use of methylamine or dimethylamine with $NaBH_3CN$ resulted in the respective reductive amination products.[27] Hertweck et al. modified leucomycin A_7 via $NaBH_3CN/ZnCl_2$-mediated reductive amination with various substituted benzylamines.[28]

In order to obtain new aminoalkyl derivatives of josamycin three reductive amination methods have been tested. Each of the methods leads to the formation of different josamycin derivatives. For the first time, the synthesis of a new type of aminoalkyl-α,β-unsaturated josamycin derivative via a 'one pot' reaction is described.

The molecular structures of josamycin (**1**) and its aminoalkyl **2**–**4** and aminoalkyl α,β-unsaturated **5**–**7** derivatives together with the atom numbering are shown in Figure 1. Our main goal initially was to obtain new aminoalkyl derivatives of josamycin via reduc-

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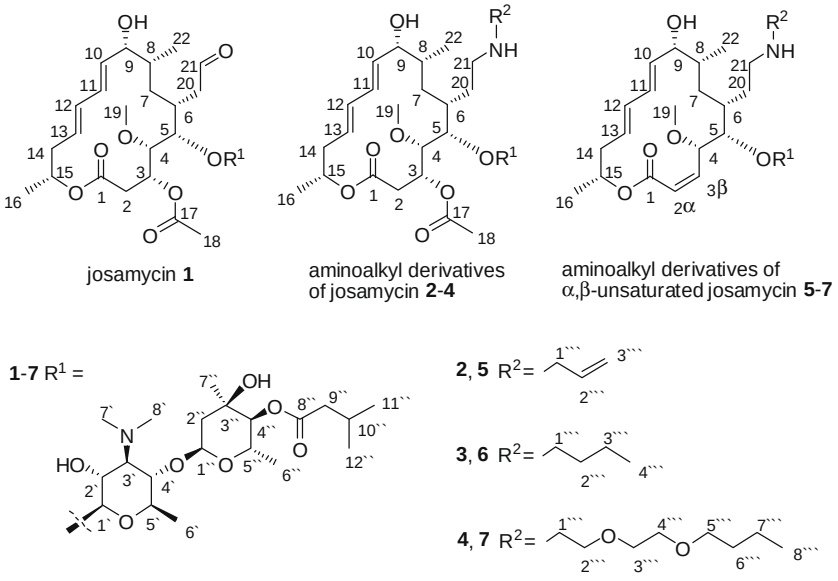

Figure 1. The structures and atom numbering of compounds **1**, **2**–**4** and **5**–**7**.

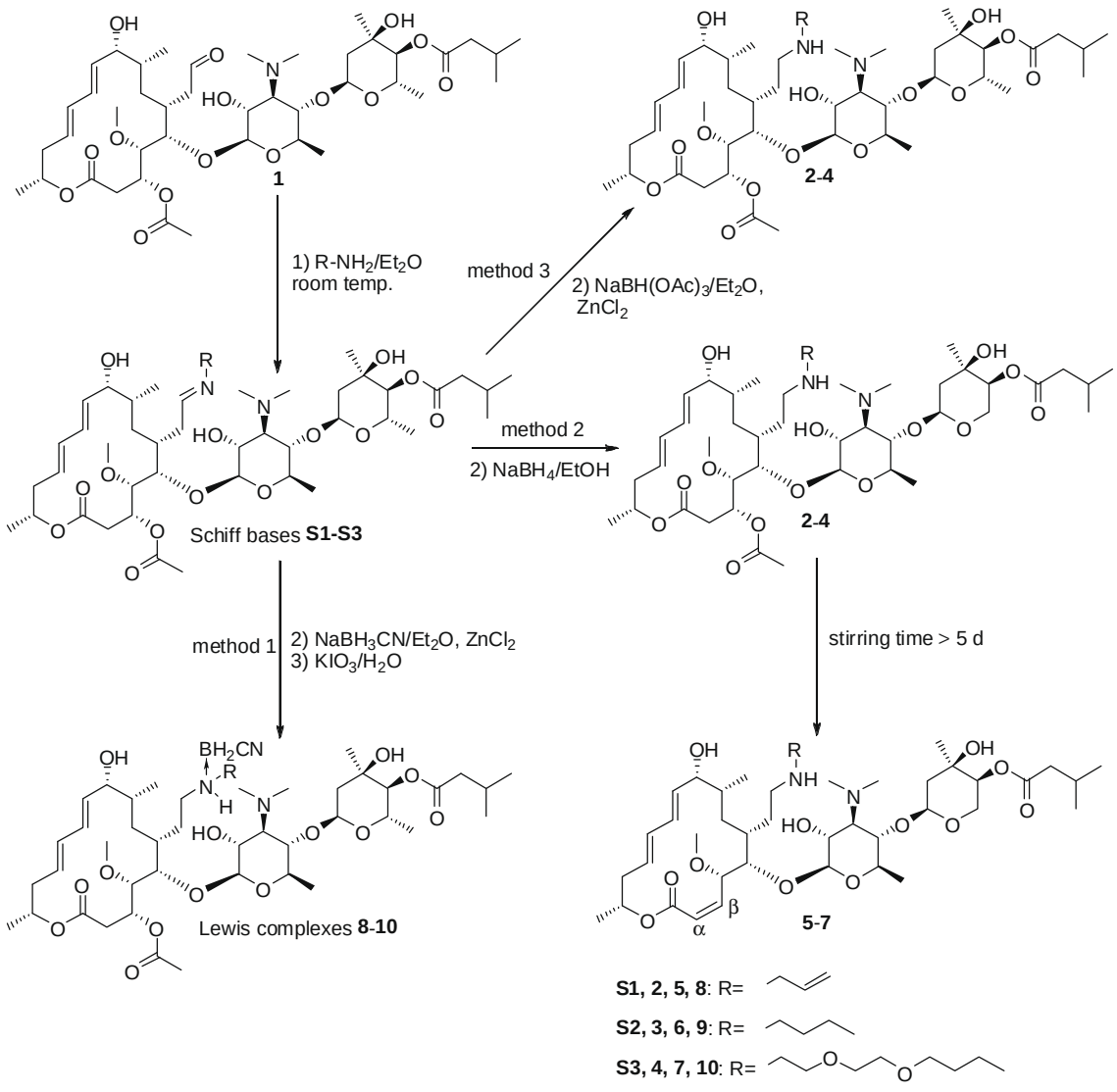

Figure 2. Methods for the reductive amination of josamycin and the products obtained.

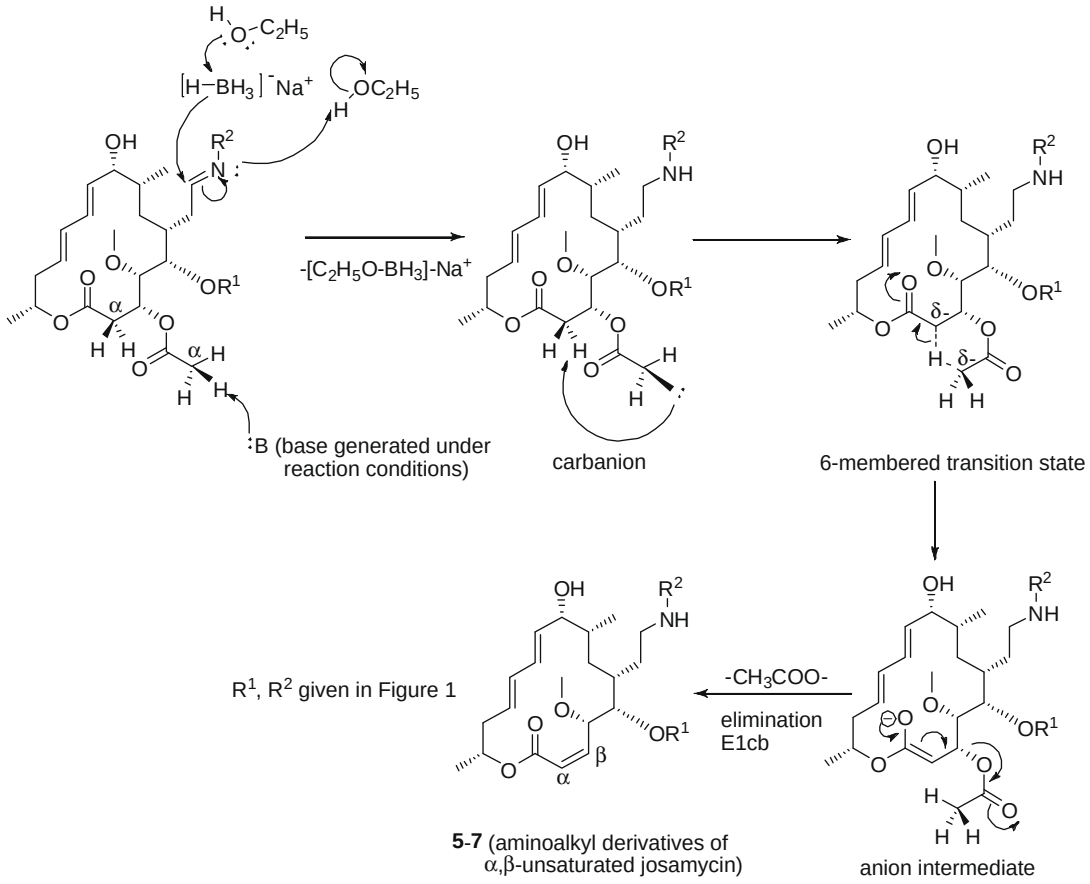

Figure 3. A plausible mechanism for the formation of **5**–**7**.

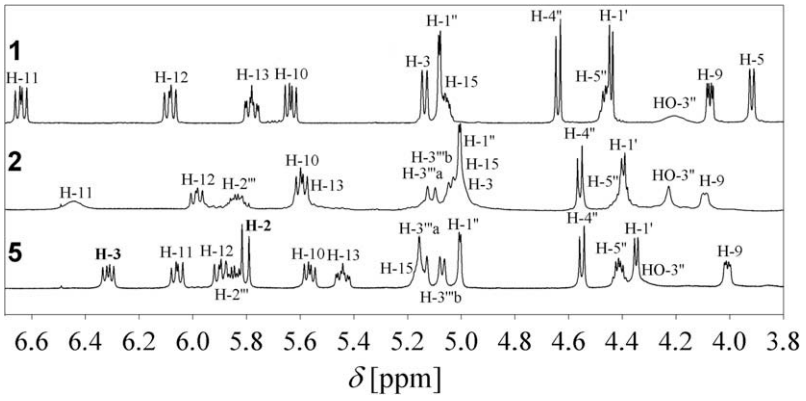

Figure 4. ^{1}H NMR spectra of compounds **1**, **2** and **5** in the range 3.8–6.7 ppm, showing the most important differences between their structures within the aglycone moiety.

tive amination using the conditions described by Hertweck et al. (method 1 in Fig. 2).[28] The addition of various alkylamines to josamycin (dissolved in ether) led to the formation of relatively unstable Schiff bases (S1–S3, Fig. 2). The ESI mass spectrum of the reaction mixture of the allylamine derivative taken after 0.5 h, showed two signals at *m/z* 829 and *m/z* 868 (see Fig. 1Sa—Supplementary data). The former signal was assigned to protonated **1**, whereas the latter to Schiff base **S1**. Additional evidence for the formation of aliphatic Schiff bases **S1**–**S3** was provided by ^{1}H NMR spectra. The spectra of the reaction mixtures after 0.5 h showed characteristic signals due to the H-21 protons in the narrow range of 7.52–7.58 ppm. In the next step, and in contrast to Hertweck,[28] the addition of $NaBH_3CN/Et_2O$ with $ZnCl_2$ and KIO_3/H_2O at room temperature surprisingly, did not yield the expected aminoalkyl derivatives of josamycin but instead another product [Fig. 1Sb (see Supplementary data) shows the ESI MS spectrum of the exclusive product formed]. The lone peak at *m/z* 909 and its associated isotopic distribution indicate that there is one boron atom in the product. Furthermore, the value of *m/z* 909 indicates the formation of a Lewis complex between the reduced Schiff bases of josamycin and the BH_2CN molecule (compounds **8**–**10**). The FT-IR spectra of Lewis complexes **8**–**10** revealed two characteristic bands at 2422 and 2200 cm^{-1} (see Fig. 2S—Supplementary data) assigned to ν(B–H) and ν(CN) stretching vibrations, respectively. In the FT-IR spectrum of $NaBH_3CN$ the ν(B–H) and ν(CN) vibrations are observed at 2181 cm^{-1} and 2343 cm^{-1}, respectively. The differences

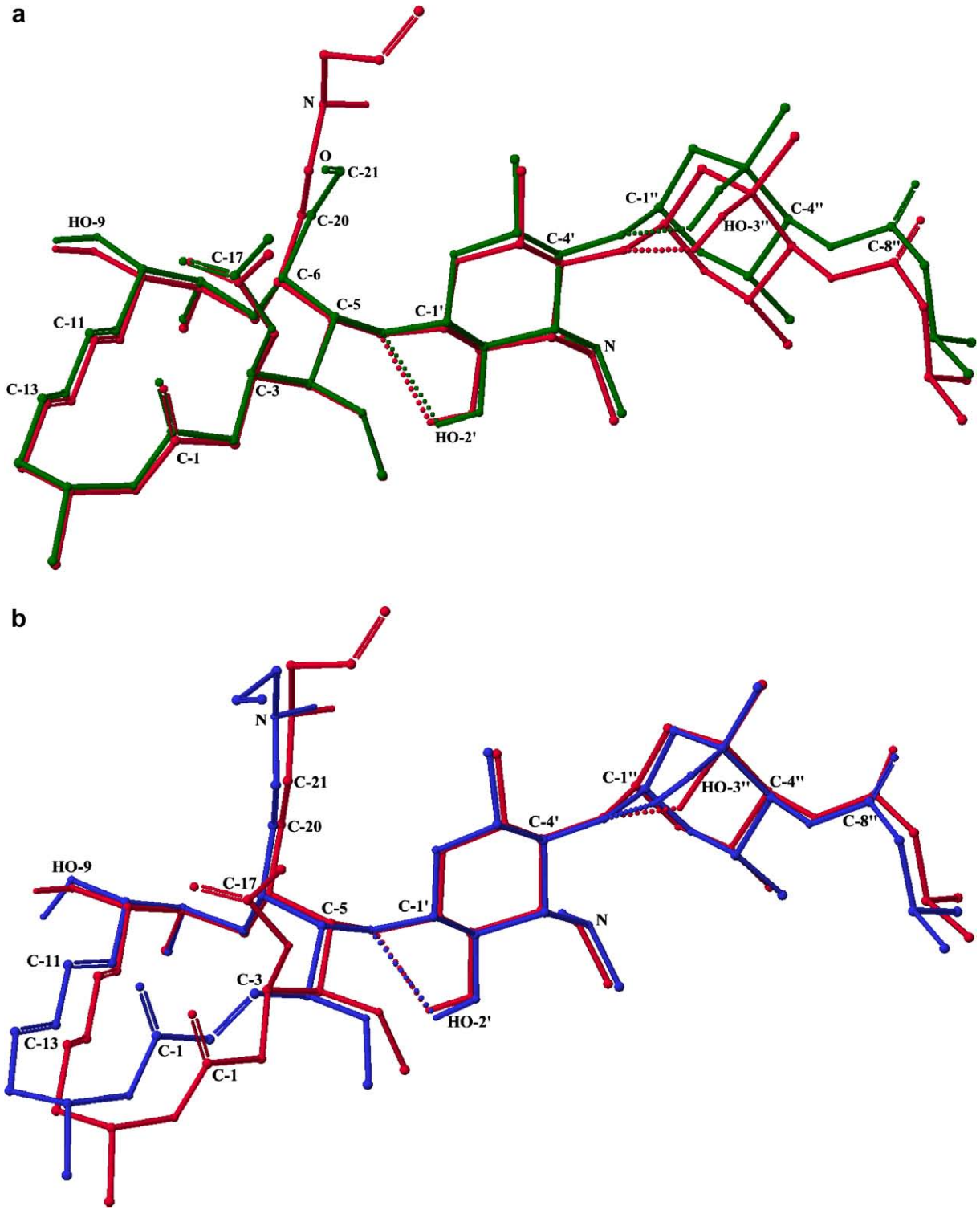

Figure 5. Comparison of the most favourable structures, calculated using the PM5 method: (a) (green line) **1** and (red line) **2**, (b) **2** (red line) and (blue line) **5**; the hydrogen atoms are omitted for clarity.

between the positions of the ν(B–H) and ν(CN) vibrations in the FT-IR spectra of $NaBH_3CN$ and products **8**–**10** indicate the formation of the respective Lewis complexes. The presence of a CN group in the Lewis complexes was proved by the analysis of the ^{13}C NMR spectra of **8**–**10** in which an additional signal at 77.9 ppm (C≡N) was detected.

Taking into account the formation of the Lewis complexes via method 1, we modified our approach to the synthesis of aminoalkyl derivatives of josamycin by using $NaBH_4$ as the reductant in ethanol (method 2 in Fig. 2). The ESI mass spectrum (see Fig. 1Sc—Supplementary data) recorded after the addition of $NaBH_4$/EtOH (5 h stirring time), showed two peaks at *m/z* 810 and 870 of which the second was assigned to the expected protonated product **2**. The *m/z* value of 810 indicates that another product (**5**) is formed as a result of elimination of one acetate group from compound **2**. The ESI MS spectrum, taken after 24 h (see

Fig. 1Sd—Supplementary data), demonstrates that the quantitative ratio of products **2** and **5** changes with increasing reaction time to the advantage of the latter. After about five days, the major products **5**–**7** were isolated, purified by column chromatography and subsequently characterized by FT-IR (Fig. 3S—Supplementary data) and NMR (Tables 1S and 2S—Supplementary data) spectra. A probable mechanism for the formation of the unexpected novel α,β-unsaturated aminoalkyl derivatives of josamycin **5**–**7** via method 2 is proposed in Figure 3. Reduction of the respective alkyl Schiff base of josamycin generates a base (e.g., EtO^-) which removes one of the acidic H-18 protons from the α position of the most accessible C-3 acetyl group. Next, the carbanion eliminates a proton α to the lactone moiety to give an anion intermediate. Finally, β-elimination of acetate (analogously to the mechanism of elimination in conjugate systems—E1cB)[29] occurs to afford products **5**–**7**.

Application of the mild reducing agent sodium triacetoxyborohydride in diethyl ether for the reductive amination (method 3 in Fig. 2) was shown to be the most efficient method for obtaining 3-acetyl aminoalkyl josamycin derivatives. The ESI mass spectra of the reaction mixtures, after 24 h stirring, showed only one characteristic signal of 100% relative abundance at *m/z* 870 for **2**, *m/z* 886 for **3** and *m/z* 974 for **4**, which were assigned to the respective $[x+H]^+$ ion, where x = **2**, **3** or **4**. Products **2**–**4** were purified by column chromatography and characterized by FT-IR (an example spectrum is shown in Fig. 3S—Supplementary data) and NMR spectra (Tables 1S and 2S—Supplementary data). The formation of products **5**, **6** and **7** was not observed using method 3.

The structures of the new aminoalkyl α,β-unsaturated josamycin derivatives **5**–**7** and the expected aminoalkyl josamycin derivatives **2**–**4** were established on the basis of spectroscopic data (see Supplementary data). A comparison of the ^1H NMR spectra of **1**, **2** and **5** (Fig. 4) shows large differences in the chemical shifts of the proton signals, especially in the aglycone part. The chemical shifts of, for example, protons H-3, H-11 and H-13 of the aglycones of **2** and **5** are evidence of the structural changes evoked by introducing an aminoalkyl substituent to josamycin at C-21 (method 3) as well as the introduction of an aminoalkyl substituent at C-21 and elimination of one acetate group from the aglycone (method 2). The structural changes are also evident in the lowest energy structures of these derivatives as calculated using the PM5 method (Fig. 5).[30]

Three types of new derivatives (**2**–**4**, **5**–**7** and **8**–**10**) were synthesized by reductive amination under different conditions. Reductive amination of josamycin with $NaBH_3CN$ under the conditions described by Hertweck surprisingly yielded stable Lewis complexes **8**–**10**. Reductive alkylation of josamycin with $NaBH_4$ gave aminoalkyl derivatives of josamycin **2**–**4** and unexpected aminoalkyl derivatives of α,β-unsaturated josamycin **5**–**7** whereas reductive amination with $NaBH(OAc)_3$ gave derivatives **2**–**4** as the exclusive products. The synthesis of new derivatives of α,β-unsaturated josamycin opens possibilities for modifications of the structure of josamycin by, for example, Michael additions.

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Supplementary data

Experimental details for the syntheses of novel compounds **2**–**7**, their characterization by ^1H and ^{13}C NMR, FT-IR and ESI MS methods together with detailed structural comparative analysis of compounds **2**–**7** are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.08.118.

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