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SYNTHESES OF *N*-ACYLISOXAZOLIDINE DERIVATIVES, RELATED TO A PARTIAL STRUCTURE FOUND IN ZETEKITOXIN AB, A GOLDEN FROG POISON

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Abstract – Syntheses of four *N*-acylisoxazolidine derivatives related to a partial structure of zetekitoxin AB were described. ^{13}C NMR spectra of these compounds could not explain an unusual chemical shift observed in the *N*-acylisoxazolidine moiety of zetekitoxin.

INTRODUCTION

In 1969, H. Mosher and co-workers reported isolation of zetekitoxin, a water soluble toxic compound from Panamanian Golden Frog, *Atelopus zeteki*.[1] Pharmacological studies revealed that the biological profile of zetekitoxin is very similar to tetrodotoxin and saxitoxin, well-known voltage-dependent Na^+ channel blocker.[2] However, since the frog is designated as an endangered species,[3] further collection of the frog is difficult and the structure remains to be clarified.

In 2004, Yamashita, Daly and co-workers proposed the structure of zetekitoxin AB to be as depicted for compound **1** (Figure 1) from extensive NMR and MS analyses using about 0.3 mg of the remaining sample.[4] The structure of zetekitoxin is closely related to saxitoxin (**2**),[5] a paralytic shellfish poison, but possesses two additional unique structural features such as a 10-membered macrolactam fused with isoxazolidine and a *N*-hydroxyl carbamate connected to guanidine through a methylene unit. Interestingly, a carbon signal observed at 156.5 ppm in the ^{13}C NMR spectrum was assigned to the carbonyl carbon at the C-13 position, a carbonyl carbon of the *N*-acylisoxazolidine. Since carbonyl carbon of the *N*-alkoxyamide is generally observed around 170-175 ppm (ex. a Weinreb amide (**3**) in Figure 1), the value of 156.5 ppm is clearly unusual. One possible explanation may be an effect of the guanidinium groups at α- and β- position of the amide carbonyl group. We found that ^{13}C-NMR spectrum of bicyclic isoxazolidine **4**[6] showed a chemical shift at 155 ppm for the carbonyl carbon, suggesting a strong effect of the nitro group at the α-position of the amide.

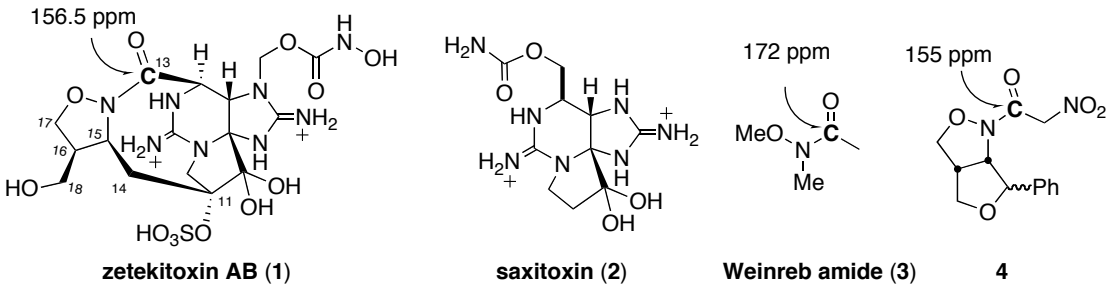


Figure 1. Structures of zetekitoxin AB and its related compounds

In order to find out the reason(s) for the unusual value of the chemical shift observed in zetekitoxin, we planed to synthesize four compounds **5**, **6**, **7**, and **8** related to the partial structure of zetekitoxin (Figure 2). ^{13}C-NMR spectra of these compounds should clarify the effect of the guanidinium group on the chemical shift of the carbonyl carbon.

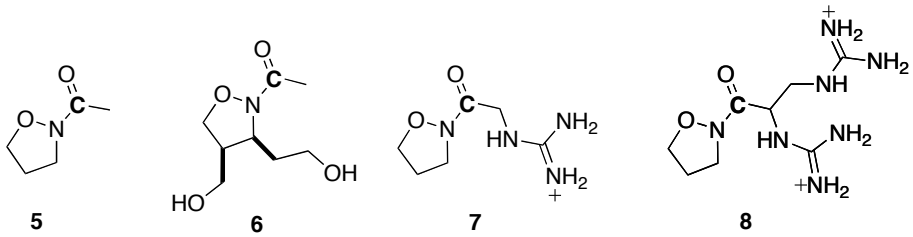
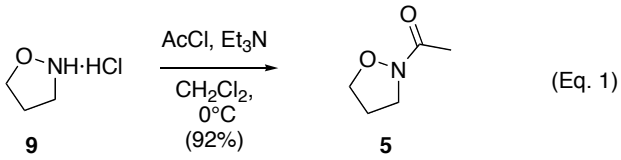


Figure 2. Structures of model compounds for the *N*-acylisoxazolidine moiety of zetekitoxin AB (**1**)

Syntheses of N-acylisoxazolidines

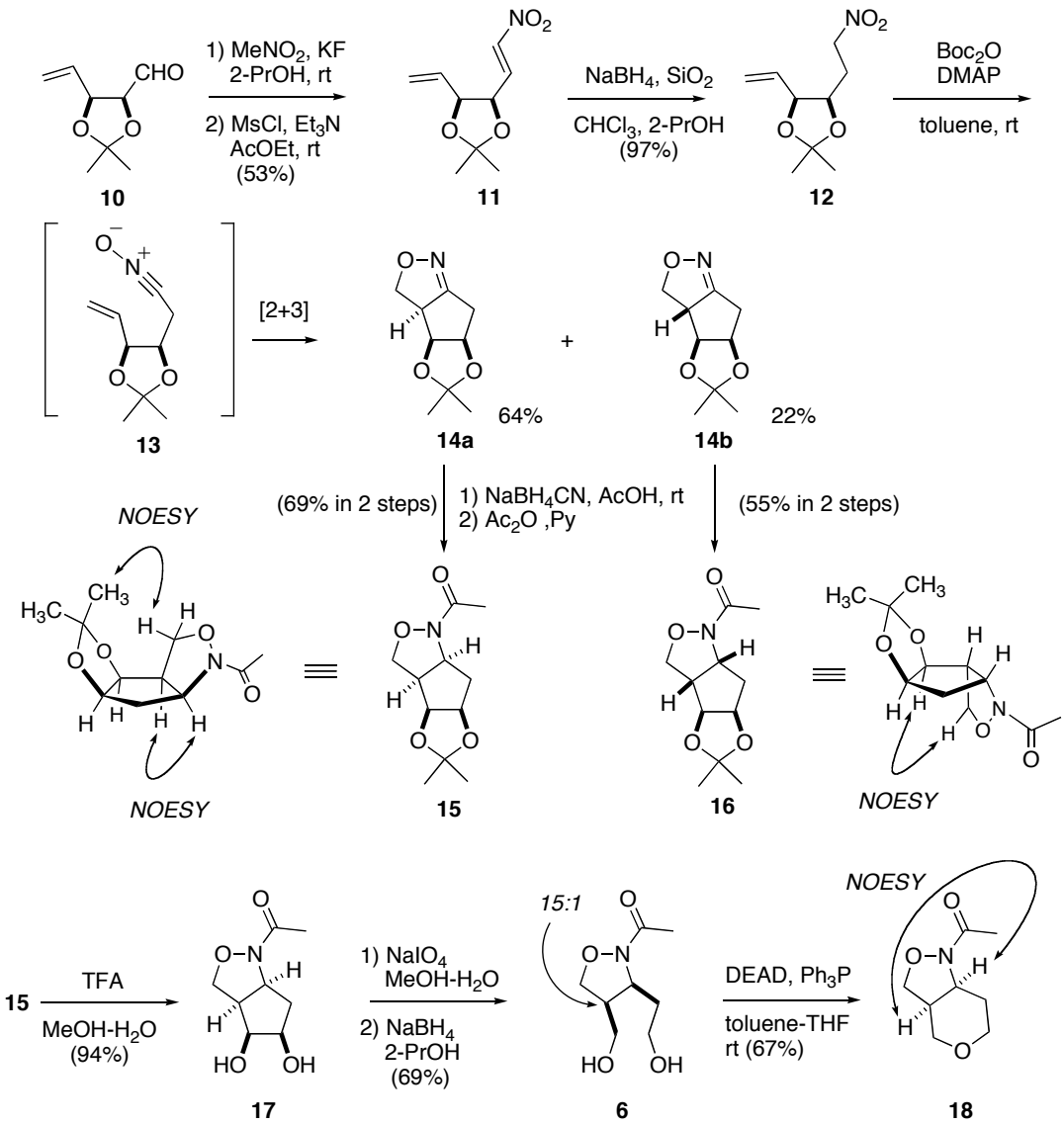
N-Acyl isoxazolidine **5**[7] was prepared by acetylation of the known isoxazolidine hydrochloride **9**[8] (Eq. 1).



(Eq. 1)

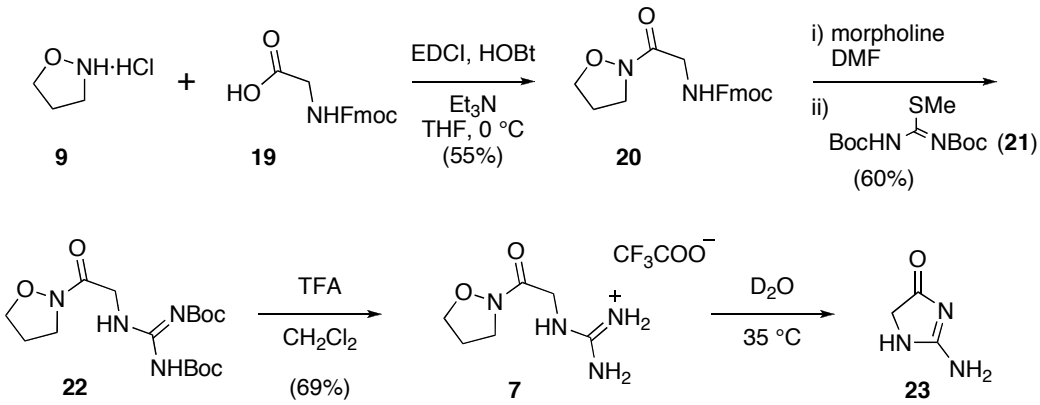
Synthesis of **6** commenced with a nitro aldol reaction of the known aldehyde **10**[9] with nitromethane in the presence of KF as a base[10] (Scheme 1). The aldol product was dehydrated under conditions using methanesulfonylation to give nitro olefin **11** in a 53% overall yield from **10**. Conjugate reduction was carried out with $NaBH_4$ in the presence of silica gel[11] to afford **12** in high yield. Upon treatment of **12** with Boc_2O and DMAP,[12] the resulting nitrile oxide **13** underwent an intramolecular 1,3-dioplar cycloaddition to give a diastereomeric mixture of **14a** and **14b** in 64 and 22% yield, respectively. After separation by silica gel column chromatography, the major product **14a** was reduced with $NaBH_3CN$ in acetic acid followed by acetylation to give *N*-acetylisoxazolidine **15**. On the other hand, the minor product **14b** was transformed to *N*-acetylisoxazolidine **16** by similar reactions. The stereochemistries of **15** and **16** were determined by NOESY spectra as shown in Scheme 1. After hydrolysis of the acetonide of major product **15** with TFA in aqueous methanol, the resulting 1,2-diol was cleaved with $NaIO_4$, and

subsequent reduction with $NaBH_4$ afforded a 15:1 mixture of diol **6**[13] and its epimer in a combined yield of 69%. The stereochemistry of **6** was confirmed by conducting NOESY experiments on tetrahydropyran **18** derived from **6** under the Mitsunobu condition.



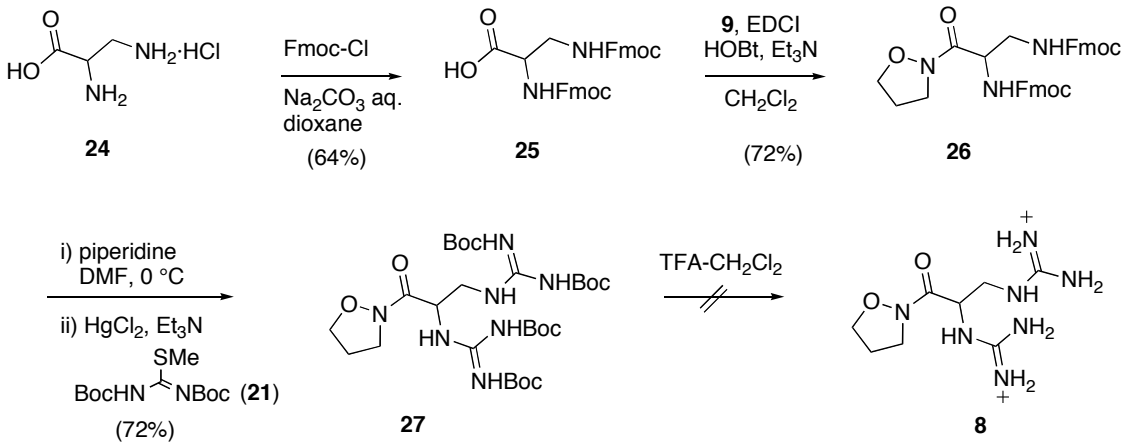
Scheme 1. Synthesis of model compound **6**

N-Acyl isoxazolidine **7** was synthesized by coupling isoxazolidine hydrochloride **9** with Fmoc-glycine **19** (Scheme 2). The coupling was carried out by EDCI and HOBt to give amide **20** in 55% yield. The Fmoc group was deprotected with morpholine and the resulting crude amine was directly treated with Boc-protected isothiourea (**21**) in one-pot to afford di-Boc guanidine **22**[14] in 60% overall yield. The two Boc groups were then deprotected with TFA in CH_2Cl_2 to give *N*-acyl isoxazolidine **7**[15] in 69% yield after recrystallization from EtOH. NMR spectra of compound **7** was measured in DMSO-d_6, since **7** forms the 5-membered guanidine **23** in D_2O via an intramolecular acylation.



Scheme 2. Synthesis of model compound **7**

N-Acyl isoxazolidine **8** would be synthesized from a commercially available *DL*-2,3-diaminopropionic acid monohydrochloride **24**. The two amino groups of **24** were protected with Fmoc groups, and the resulting **25** was coupled with isoxazolidine hydrochloride **9** in the presence of EDCI and HOBt to give amide **26** in 72% yield. Deprotection of the Fmoc groups was followed by guanidinylation with Boc-protected isothiourea (**21**) in the presence of $HgCl_2$ to afford bis-di-Boc guanidine **27**.[16] However, the formation of guanidine **8** by deprotection of the four Boc groups under acidic conditions was unsuccessful, probably due to a similar intramolecular acylation of guanidine as encountered in the synthesis of **7**.

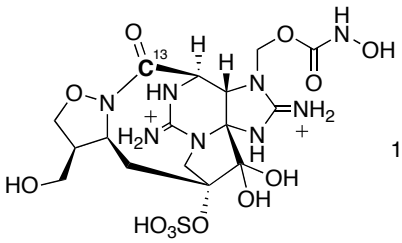
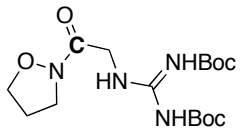
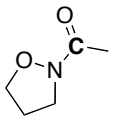
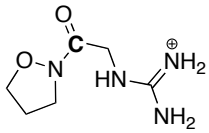
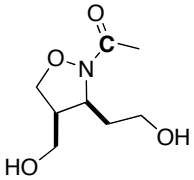
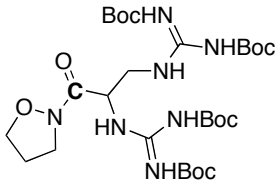


Scheme 3. Synthesis of model compound **8**

With the synthetic compounds **5**, **6**, **7**, and **27** in our hands, ^{13}C NMR chemical shifts of the amide carbonyl group of these compounds were compared with zetekitoxin AB (Table 1). Although the model compound **8** could not be prepared, ^{13}C NMR spectrum of **27** was used instead of **8** for the comparison, because the Boc groups of the guanidine have little influence on the chemical shifts of the amide carbon, judging from comparison between **22** and **7** (Table 1). The model compounds **5** and **22** show two

different amide absorptions, respectively, due to the existence of amide rotamers. Chemical shifts for the amide carbon of **5** and **6** were observed in the usual range for amide carbons. Chemical shifts for the amide carbon in **7** and **27** were shifted upfield by about 5 ppm due to the inductive effect of guanidine at the α-position. The guanidine function at β-position has little influence on the amide carbon.

Table 1. ^{13}C NMR chemical shifts for the amide carbonyl carbon of **1**, **5**, **6**, **22**, **7**, and **27**

compound	chemical shift of amide carbon (ppm)	compound	chemical shift of amide carbon (ppm)
 **zetekitoxin AB** (**1**)	156.5	 **22**	166.0, 168.3[a, c]
 **5**	170.1, 173.3[a]	 **7**	168.3[b]
 **6**	172.7[a]	 **27**	167.0[b]

[a] ^{13}C NMR was measured in $CDCl_3$. [b] ^{13}C NMR was measured in DMSO-d_6. [c] Amide carbons were assigned from HMBC correlation between the methylene proton on isoxazolidine and the carbonyl carbon.

CONCLUSION

We have synthesized *N*-acyl isoxazolidine derivatives **5**, **6**, **7** and **27**, and measured ^{13}C NMR spectra of these synthetic compounds. Comparison of the ^{13}C NMR chemical shift of **5**, **6**, **7**, **27** and zetekitoxin led us to conclude that the unusual chemical shift of the C-13 of zetekitoxin AB could not be rationalized only by the substituent effect of the guanidinium groups. The chemical shift at the C-13 might be explained by unknown factor(s) derived from the rigid 10-membered macrolactam structure of zetekitoxin. Further efforts to find the factor are in progress in our laboratory.

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7. Compound (**5**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.32 (2H, quintet J = 7 Hz, CH_2-CH_2-CH_2), 3.71 (2H, t, J = 7 Hz, N-CH_2), 3.97 (2H, t, J = 7 Hz, O-CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.2, 20.7, 27.5, 42.9, 69.1, 170.7, 173.3. Anal. Calcd for $C_5H_9NO_2$: C, 52.16; H, 7.88; N, 12.17. Found: C, 52.16; H, 7.97; N, 12.38.
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11. A. K. Sinhababu and R. T. Borchardt, *Tetrahedron Lett.*, 1983, **24**, 227.
12. Y. Basel and A. Hassner, *Synthesis,* 1997, 309.
13. Compound (**6**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (1H, m, C$H_A$$H_B$-$CH_2$-O), 1.86 (1H, dddd, J = 14, 12, 5, 3 Hz, $CH_A$$H_B$-$CH_2$-O), 2.16 (3H, s, Ac), 2.94 (1H, m, -CH-), 3.58 (1H, td, J = 12, 3 Hz, CH_2-C$H_A$$H_B$), 3.69 (1H, m, CH-$CH_A$$H_B$), 3.70 (1H, dd, J = 10.5, 8 Hz, CH-C$H_A$$H_B$-OH), 3.83 (1H, dd, J = 10.5, 6 Hz, CH-$CH_A$$H_B$-OH), 4.04 (2H, d, J = 6 Hz, CH_2-O), 4.67 (1H, ddd, J = 12, 8, 6 Hz, N-CH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.1, 31.2, 46.2, 54.2, 58.6, 59.7, 72.0, 172.7. HRMS (FAB) for $C_8H_{16}NO_4$ (M+H), calcd 190.1079, found 190.1066. Compound (**6**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (1H, m, C$H_A$$H_B$-$CH_2$-O), 1.86 (1H, dddd, J = 14, 12, 5, 3 Hz, $CH_A$$H_B$-$CH_2$-O), 2.16

(3H, s, Ac), 2.94 (1H, m, -C*H*-), 3.58 (1H, td, *J* = 12, 3 Hz, CH_2-C$H_A$$H_B$), 3.69 (1H, m, CH-$CH_A$$H_B$), 3.70 (1H, dd, *J* = 10.5, 8 Hz, CH-C$H_A$$H_B$-OH), 3.83 (1H, dd, *J* = 10.5, 6 Hz, CH-$CH_A$$H_B$-OH), 4.04 (2H, d, *J* = 6 Hz, CH_2-O), 4.67 (1H, ddd, *J* = 12, 8, 6 Hz, N-C*H*). ^{13}C NMR (75 MHz, $CDCl_3$) δ 20.1, 31.2, 46.2, 54.2, 58.6, 59.7, 72.0, 172.7. HRMS (FAB) for $C_8H_{16}NO_4$ (M+H), calcd 190.1079, found 190.1066.

14. Compound (**22**): ^{1}H NMR (400 MHz, $CDCl_3$) **major peak**; δ 1.50 (18H, s, Boc x 2), 2.33 (2H, quintet *J* = 7 Hz, CH_2-CH_2-CH_2), 3.75 (2H, t, *J* = 7.5 Hz, N-CH_2), 3.99 (2H, t, *J* = 7 Hz, O-CH_2), 4.34 (2H, d, *J* = 4.5 Hz, CO-CH_2), 9.08 (1H, m, N*H*), 11.40 (1H, br s, N*H*). **minor peak**; δ 1.51 (18H, s, Boc x 2), 3.45 (2H, t, *J* = 5 Hz, CH_2-CH_2-CH_2), 3.67 (4H, m, CH_2-N, CH_2-O), 4.25 (2H, d, *J* = 4 Hz, CH_2-NH), 9.42 (1H, m, N*H*), 11.39 (1H, m, N*H*). ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.3, 28.0, 28.2 (two peaks), 42.3, 42.5, 42.9, 43.5, 44.8, 66.3, 66.7, 69.4, 79.3, 79.7, 83.1, 83.3, 152.6, 152.7, 155.4, 155.8, 162.8, 163.1, 166.0, 168.2. Anal. Calcd for $C_{16}H_{28}N_4O_6$: C, 51.60; H, 7.58; N, 15.04. Found: C, 51.60; H, 7.50; N, 14.89.
15. Compound (**7**): ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.26 (2H, quintet, *J* = 7 Hz, CH_2-CH_2-CH_2), 3.69 (2H, t, *J* = 7 Hz, N-CH_2), 3.97 (2H, t, *J* = 7 Hz, O-CH_2), 4.10 (2H, d, *J* = 5 Hz, CO-CH_2), 7.20-7.72 (5H, m, N*H*). ^{13}C NMR (100 MHz, DMSO-d_6) δ 27.3, 42.4, 43.9, 69.4,157.7, 168.3. HRMS (FAB) for $C_6H_{13}N_4O_2$ (M+H), calcd 173.1039, found 173.1078.
16. Compound (**27**): ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.38 (18H, s, Boc x 2), 1.45 (9H, s, Boc), 1.46 (9H, s, Boc), 2.30 (2H, m, CH_2C$H_2$$CH_2$), 3.47 (1H, m, N-C$H_A$$H_B$), 3.60-3.82 (3H, m, N-C$H_A$$H_B$, C$H_2$NH), 4.10 (1H, m, O-C$H_A$$H_B$), 4.17 (1H, m, O-$CH_A$$H_B$), 5.06 (1H, m, C*H*), 8.42 (1H, br s, N*H*), 8.76 (1H, br s, N*H*), 11.41 (1H, br s, N*H*), 11.45 (1H, br s, N*H*). ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.3, 25.6, 27.3, 27.7, 27.8, 28.1, 30.6, 31.5, 43.7, 50.9, 51.0, 55.1, 65.1, 69.5, 78.3, 78.4, 78.5, 87.0, 83.1, 83.2, 83.3, 148.5, 151.2, 151.8, 151.9 (two peaks), 155.0, 156.2, 162.8, 162.9, 163.0, 163.1, 167.0. HRMS (FAB) for $C_{28}H_{50}N_7O_{10}$ (M+H), calcd 644.3619, found 644.3593.