Two New Spermidine Alkaloids from *Chisocheton weinlandii*

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The investigation of the MeOH extract of the leaves of *Chisocheton weinlandii* HARMS (Meliaceae) revealed two new open-chain spermidine alkaloids, chisitine 1 (**1**) and chisitine 2 (**2**). Their structures were elucidated by NMR spectroscopy, tandem-mass spectrometry, and independant syntheses (*Scheme 3*). Detailed MS/MS fragmentation pathways are discussed for both compounds based on H/D exchange and ^{18}O-labeling experiments (*Schemes 1* and *2*).

1. Introduction. – Polyamine alkaloids are found in a large variety of natural sources including plants and insects. These natural products are classified into two major categories depending on whether the structure is cyclic or open-chained [1]. In general, such polyamine alkaloids are composed of a putrescine (= butane-1,4-diamine), spermidine (= *N*-(3-aminopropyl)butane-1,4-diamine), or spermine (= *N*,*N*′-bis(3-aminopropyl)butane-1,4-diamine) backbone that carries one or more substituents at the N-atoms, mostly acyl groups. Their determination was usually performed by a combination of several analytical techniques, the methods of choice being mass spectrometry combined with NMR spectroscopy.

However, NMR spectroscopy often does not securely assess the exact framework of the polyamine portion within these alkaloids because of signal overlap of the methylene protons. Furthermore, acyl-substituted polyamines display an additional range of complexity in their NMR spectra due to s-*cis*/s-*trans* isomerism of the amide functions. The NMR spectra of such isomer mixtures, exhibiting multiple signals for the NMR active nuclei, always leave doubt about whether the sample was pure or not.

Mass spectrometry – in combination with tandem-mass spectrometry (MS/MS) – is gaining more and more importance in the identification and characterization of polyamine alkaloids. Due to their high basicity and low volatility, polyamines are excellent candidates for mass-spectrometric identification by means of electrospray ionization (ESI-MS). This method allows to delimit the number of N-atoms present in the molecule. In addition, MS/MS experiments can provide precious information on the polyamine backbones and on the attachment sites of substituents. Furthermore, as compared to NMR spectroscopy, data acquisition for MS is much faster and requires considerably smaller amounts of material.

In connection with our ongoing search for new polyamine alkaloids from plants and animal sources, we were interested in the MeOH extracts of the leaves of *Chisocheton*

weinlandii HARMS (Meliaceae). This tree is distributed throughout southern Irian Jaya (Papua New Guinea, Indonesia). Around fifty putrescine alkaloids have already been characterized from species belonging to the plant family Meliaceae [1]. Several *Chisocheton* species have been screened for their natural-product content; among them, *Chisocheton paniculatus* HIERN has been intensively investigated. Five apotirucallol derivatives and four tetranortriterpenoids from the wood and seeds of *C. paniculatus* were identified [2]. Four meliacin-type compounds were found in the fruits [3], and five protolimonoids (called paniculatin B, C, D, G, and H) and one limonoid (named arunachalin) were isolated and identified from the mature wood of the plant [4]. For *C. weinlandii*, on the other hand, no study of the chemical constituents is found in the literature. Only some insecticidal activity was reported for extracts of its leaves and twigs [5]. These extracts showed inhibition to larval growth of the variegated cutworm *Peridroma saucia* HÜBNER (Noctuinidae). We have now studied the constituents of the leaves of the plant and isolated the two new spermidine-type alkaloids **1** and **2** (see *Fig. 1*). Their structures were established on the basis of NMR spectroscopy, tandem-mass spectrometry, and comparison with their synthetic equivalents.

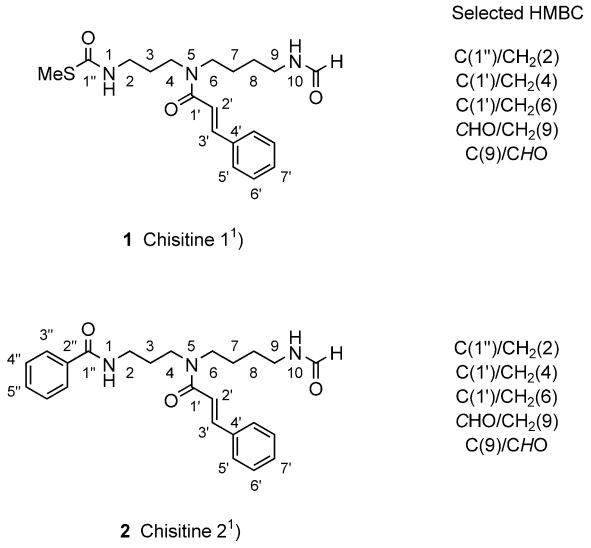


Fig. 1. *Structure and selected HMBC data of chisitine 1* (**1**) *and 2* (**2**)

2. Results. – 2.1. *Isolation*. Extraction of the crushed leaves of *C. weinlandii* with MeOH and distribution of the extract between H_2O and AcOEt yielded a crude mixture that was separated by column chromatography (silica gel). With the eluent $CHCl_3$/MeOH 5:1, a mixture was obtained that was further subjected to semi-prep. reversed-phase HPLC, resulting in the isolation of two pure compounds named

[1]) Arbitrary numbering; for systematic name, see *Exper. Part.*

chisitine 1 (**1**) and chisitine 2 (**2**) (*Fig. 1*). To the best of our knowledge, these are the first spermidine alkaloids isolated from a Meliaceae species.

2.2. *Structure Elucidation*. Chisitine 1 (**1**) shows in its ESI-MS a series of three peaks at *m/z* 378 ($[M+H]^+$), 400 ($[M+Na]^+$), and 416 ($[M+K]^+$) that allow the assignment of its relative molecular mass as 377. The value suggests the presence of an odd number of N-atoms in the molecule. The ^{1}H- and ^{13}C-NMR spectra of **1**, showing a 1:1 ratio of two isomers (for data, see isomers **1a**, and **1b** in *Tables 1* and *2*), indicate the presence of a cinnamoyl (=(2*E*)-3-phenylprop-2-enoyl) substituent. After detailed analysis of further NMR and MS data, the structure of chisitine 1 (**1**) was elucidated as *S*-methyl [3-{[4-(formylamino)butyl][(2*E*)-3-phenylprop-2-enoyl]amino}propyl]carbamothioate. This compound was synthesized, and the synthetic product was found to be identical with the natural product with respect to all its spectroscopic data (see *Sect. 2.4*).

Table 1. *^{1}H-NMR Data* (500.1 or 600.1 MHz, (D_6)DMSO) *of Chisitines 1* (**1**) *and 2* (**2**)[1]). δ in ppm, *J* in Hz.

	1[a])		**2**[b])	
	Isomer **1a**	Isomer **1b**	Isomer **2a**	Isomer **2b**
H–N(1)	8.23 (br. *s*)	8.11 (br. *s*)	8.49–8.47 (*m*)	8.57–8.55 (*m*)
CHO	8.00 (br. *s*)[c])		8.00 (br. *s*)[c])	
H–N(10)				
H–C(3″)	–		7.87 (*d*, *J* = 7.4)	7.85 (*d*, *J* = 7.4)
H–C(5′)	7.70–7.68 (*m*)[c])		7.71 (*d*, *J* = 7.4)	7.58 (*d*, *J* = 7.1)
H–C(5″)	–		7.54–7.45 (*m*)[c])	
H–C(3′)	7.49 (br. *d*, *J* ≈ 15.2)[c])			
H–C(4″)	–			
H–C(6′)	7.44–7.36 (*m*)[c])		7.44–7.27 (*m*)[c])	
H–C(7′)				
H–C(2′)	7.10 (*d*, *J* = 15.4)	7.05 (*d*, *J* = 15.4)	7.13 (*d*, *J* = 15.4)	7.05 (*d*, *J* = 15.4)
CH_2(4)	3.49–3.45 (*m*)[c])	3.32–3.29 (*m*)[c])	3.55–3.50 (*m*)[c])	3.44–3.42 (*m*)[c])
CH_2(6)	3.32–3.29 (*m*)[c])	3.49–3.45 (*m*)[c])	3.38–3.34 (*m*)[c])	3.55–3.50 (*m*)[c])
CH_2(2)	3.21–3.15 (*m*)[c])	3.13–3.08 (*m*)[c])	3.38–3.34 (*m*)[c])	3.29–3.26 (*m*)[c])
CH_2(9)	3.13–3.08 (*m*)[c])		3.13–3.08 (*m*)[c])	
MeS	2.21, 2.20 (2*s*)[d])		–	
CH_2(3)	1.74–1.71 (*m*)	1.68–1.65 (*m*)	1.87–1.84 (*m*)	1.79–1.77 (*m*)
CH_2(7)	1.54–1.36 (*m*)[c])		1.57–1.49 (*m*)[c])	
CH_2(8)			1.38–1.41 (*m*)	1.48–1.43 (*m*)

[a]) 500.1 MHz. [b]) 600.1 MHz. [c]) Signals of isomers **a/b** overlapped. [d]) Not distinguishable.

In the ^{1}H-NMR spectrum of **1**, the two *d* at δ 7.10 and 7.05 and the broad *d* at δ 7.49 (^3J = 15.4) are attributed to the olefinic protons H–C(2′) and H–C(3′) of the two isomeric forms **1a** and **1b**[1]). The corresponding ^{13}C-NMR signals for C(2′) and C(3′) are found at δ 118.4 and 118.3, and 141.3 and 141.2 and secured by HSQC. The signals at δ(C) 161.0 and 160.9 (for **1a** and **1b**) and the broad *s* at δ(H) 8.00 suggest the presence of a formamide function [6]. A third substituent is identified by the signal at δ(H) *ca.* 2.20 (3 H), which correlates to the *q* (determined by DEPT) at δ(C) 11.5 (two overlapping signals). These signals are attributed to the unusual MeSC(O)N residue. The analytical data are in accordance with other carbamothioate-containing alkaloids [7][8]. The presence of the MeSC(O)N group is further supported by the MS/MS results: the intense fragment ion **l** (*m/z* 330), corresponding to the loss of 48 Da from the quasi-molecular ion ($[M+H]^+$) at *m/z* 378, indicates an easy loss of MeSH (see *Fig. 3,a*, below). ^{1}H-NMR Signals registered between δ 1.36 and 3.49 (integrating for 14 H) and the ^{13}C-NMR signals found between δ 26.3 and 46.8 (all *t*) suggest a spermidine

Table 2. *^{13}C-NMR Data* (125.8 or 150.9 MHz, (D_6)DMSO) *of Chisitines 1* (**1**) *and 2* (**2**)[1]). δ in ppm, *J* in Hz.

	1[a])		**2**[b])	
	Isomer **1a**	Isomer **1b**	Isomer **2a**	Isomer **2b**
C(1″)	166.2 (*s*)	166.0 (*s*)	166.4 (*s*)	166.1 (*s*)
C(1′)	165.2 (*s*)	164.9 (*s*)	165.3 (*s*)	164.9 (*s*)
CHO	161.0 (*d*)	160.9 (*d*)	161.0 (*d*)	160.9 (*d*)
C(3′)	141.3 (*d*)	141.2 (*d*)	141.3 (*d*)	141.2 (*d*)
C(4′)	135.1 (*s*)	135.0 (*s*)	135.1(*s*)	135.0 (*s*)
C(2″)	–	–	134.7 (*s*)	134.5 (*s*)
C(5″)	–	–	131.1 (*d*)	131.0 (*d*)
C(7′)	129.5 (*d*)[c])		129.5 (*d*)	129.4 (*d*)
C(6′)	128.7 (*d*)[c])		128.7 (*d*)	128.6 (*d*)
C(4″)	–	–	128.3 (*d*)[c])	
C(5′)	127.9 (*d*)[c])		128.0 (*d*)	127.8 (*d*)
C(3″)	–	–	127.1 (*d*)	127.0 (*d*)
C(2′)	118.4 (*d*)	118.3 (*d*)	118.5 (*d*)	118.4 (*d*)
C(6)	45.4 (*t*)	46.8 (*t*)	45.4 (*t*)	46.8 (*t*)
C(4)	44.7 (*t*)	43.6 (*t*)	45.0 (*t*)	43.6 (*t*)
C(2)	38.1 (*t*)	38.5 (*t*)	36.7 (*t*)	36.9 (*t*)
C(9)	36.8 (*t*)	36.6 (*t*)	36.8 (*t*)	36.6 (*t*)
C(3)	29.5 (*t*)	27.7 (*t*)	29.6 (*t*)	27.7 (*t*)
C(8)	24.9 (*t*)	26.7 (*t*)	25.0 (*t*)	26.7 (*t*)
C(7)	26.6 (*t*)	26.3 (*t*)	26.6 (*t*)	26.3 (*t*)
MeS	11.5 (*q*)[c])		–	

[a]) 125.8 MHz. [b]) 150.9 MHz. [c]) Signals of isomers **a/b** overlapped.

backbone for the compound. The sites of attachment of the three substituents at the polyamine core, as well as the detailed assignments of the signals, were finally established on the basis of the MS/MS analysis (see *Sect. 2.3*) as well as on the basis of the HMBC experiment (selected HMBC data in *Fig. 1*).

The second compound, chisitine 2 (**2**), shows three adduct ions in the ESI-MS at *m/z* 408 ($[M+H]^+$), 430 ($[M+Na]^+$), and 446 ($[M+K]^+$), consistent with a relative molecular mass of 407. This indicates that chisitine 2 also contains an odd number of N-atoms. The ^{1}H- and ^{13}C-NMR spectra of this alkaloid show the presence of the same 1:1 ratio of two isomers as found for **1** (for data, see isomers **2a** and **2b** in *Tables 1* and *2*). Moreover, the data also indicate the presence of an acyl-substituted spermidine, carrying the cinnamoyl and formyl moieties, but not the methyl carbamothioate unit. Since the sites of attachment of the groups are identical to those found for chisitine 1, the only difference between **2** and **1** concerns the nature of one substituent. Based on further spectral data, the structure of **2** was determined to be *N*-{3-{[4-(formylamino)butyl][(2*E*)-3-phenylprop-2-enoyl]amino}propyl}benzamide and confirmed by comparison with the analytical data of the synthetic chisitine 2 (*Sect. 2.4*).

The additional signals in the aromatic region of the ^{13}C-NMR spectra of **2** at δ 134.7 and 134.5 (2 *s*, C(2″)), 131.1 and 131.0 (2 *d*, C(5″)), 128.3 (2 overlapping *d*, C(4″)), and 127.1 and 127.0 (2 *d*, C(3″)) are assigned to a benzoyl group [9][1]). The replacement of the MeSCO in **1** by the PhCO group in **2** is consistent with the difference of 30 Da in their relative molecular mass. The sites of attachment of the substituents at the spermidine core for both isomers were established on the basis of a detailed MS/MS analysis (see *Sect. 2.3*) and of the HMBC spectra (see data in *Fig. 1*).

2.3. *Tandem Mass Spectrometry of Chisitines* **1** *and* **2**. A special problem arises in the correct placing of the two substituents (formyl and (methylthio)carbonyl in case of **1** and formyl and benzoyl in case of **2**) at the two primary N-atoms of the spermidine core. The question that arises is whether the amino groups are separated by three or four CH_2 residues from the third central N-atom. In addition to our earlier investigations in this field of isomerism [10–12], we have studied very carefully this problem. Indeed, it seems necessary to determine the general characteristic MS fragmentation of this kind of compound, because similar natural products isolated in much smaller amounts have to be investigated in the future.

Compounds **1** and **2** were examined by ESI-MS/MS on a quadrupole ion-trap mass spectrometer. The two compounds produce two generations of daughter ions (MS^2 and MS^3) on the basis of which fragmentation mechanisms are proposed. The aim of their detailed elucidation is to detect characteristic fragmentation pathways that are diagnostic for the structures proposed for **1** and **2**. Characteristic ions could indicate the location of the substituents as well as the sequence of the polyamine backbone (PA34 or PA43). The MS/MS data of compound **2** contain more informative ion responses allowing the postulation of general fragmentation pathways, and are, thus, discussed first.

Upon MS^2, quasi-molecular ion $[\mathbf{2}+H]^+$ at *m*/*z* 408 yields the four important fragment ions **a**, **d**, and **e**/**e′** at *m*/*z* 390, 278, and 260 (*Fig. 2*,*a*). These ions are believed to arise from two major *Pathways A* and *B* (*Scheme 1*). Following *Pathway A*, $[\mathbf{2}+H]^+$ could lose the cinnamoyl moiety (−130 Da) to form fragment ion **d** (*m*/*z* 278). This suggested four-center cleavage has already been reported for *N*-coumaroylputrescine [12]. Fragment ion **d** could lose H_2O by reaction of the secondary amine ($N(5)^1$)) with either one of the two amide groups to form cyclic fragment ions **e** (1,6-cyclization) or **e′** (1,7-cyclization), detected at *m*/*z* 260 as the base peaks in MS^2. Analogous dehydrations have been observed previously with peptides [13]. The cyclization **d** → **e** and **e′** is established by investigation of the labeled analog [^{18}O]-**2** (*Fig. 2*,*c*). The MS^2 of [[^{18}O]-$\mathbf{2}+H]^+$ (*m*/*z* 410) reveals a doublet of peaks corresponding to **e** at *m*/*z* 260 and [^{18}O]-**e′** (*m*/*z* 262), which attests that both proposed pathways are followed concurrently.

Isolation and further fragmentation (MS^3) of **e** and **e′** at *m*/*z* 260 yield the four new informative ions **g**, **h**, **j**, and **k** at *m*/*z* 162, 161, 105, and 99, respectively. Probably ion **e′** leads to two cyclic fragment ions **g** (*m*/*z* 162) and **k** (*m*/*z* 99), and to the benzoyl ion **j** (*m*/*z* 105). These three ions are established to originate from **e′** by investigation of [^{18}O]-**2** (see *Fig. 2*,*c*): the corresponding signals for **g** and **j** are at *m*/*z* 164 (+2 Da) and 107 (+2 Da), respectively, whereas ion **k** is at the same *m*/*z* value 99 (see *Scheme 1*). Fragment ion **g** (*m*/*z* 162) reveals that the benzoyl group is linked to the trimethylene portion of the spermidine, while **k** (*m*/*z* 99) attests that the formyl moiety is located at the end of the tetramethylene unit.

Fragmentation of the six-membered ion **e** (*m*/*z* 260) leads to the formation of ion **h** at *m*/*z* 161, additionally supported by the MS^3 (410 → 260) of 'pure' **e** in the spectrum of [^{18}O]-**2** (*Fig. 2*,*c*). The diagnostic ions **g**, **k**, and **h**, therefore, justify the structure of chisitine 2 (**2**) independently of the NMR spectra.

The second important fragmentation *Pathway B* starts with the direct dehydration of quasi-molecular ion $[\mathbf{2}+H]^+$ giving rise to a signal at *m*/*z* 390 ($[\mathbf{2}+H-18]^+$). Again, the loss of H_2O is explained by the formation of a cyclic structure, fragment ion **a**

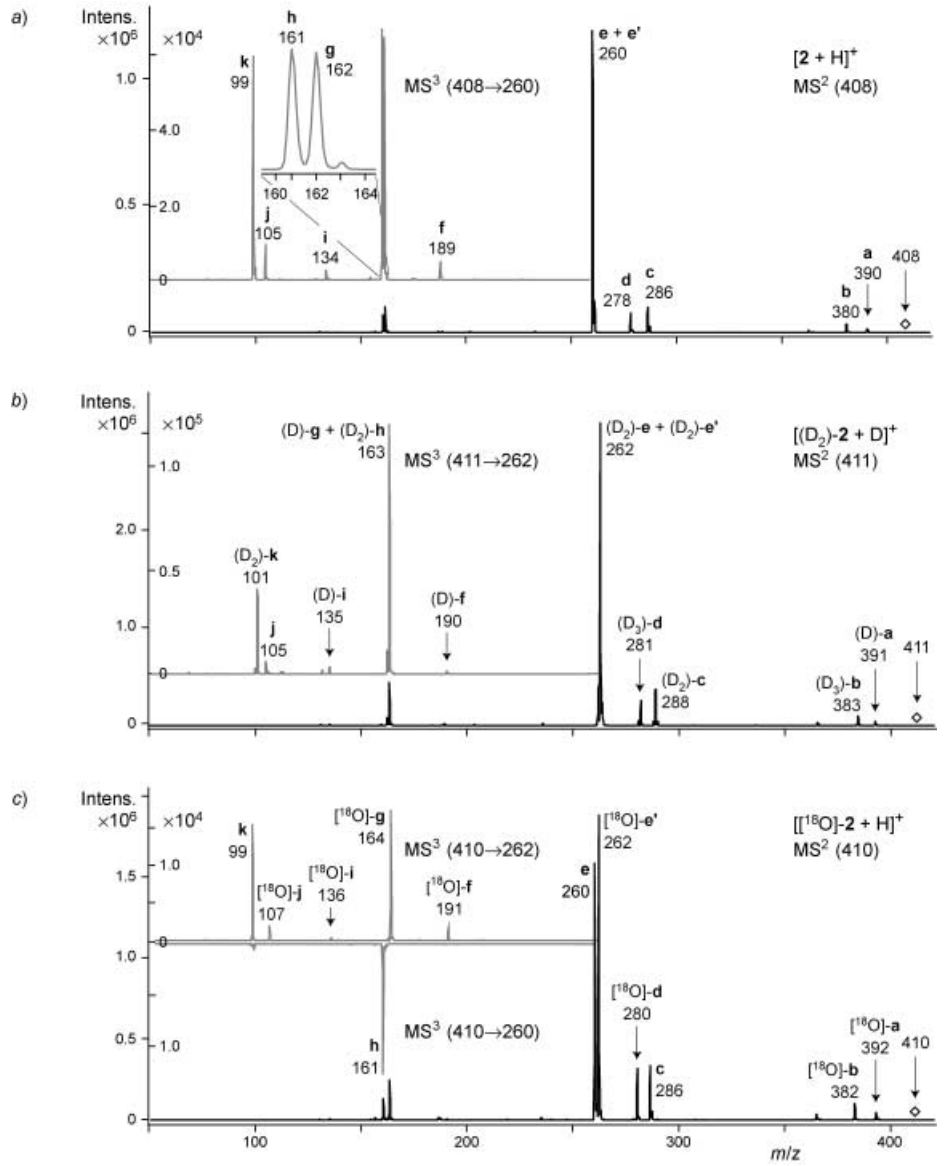
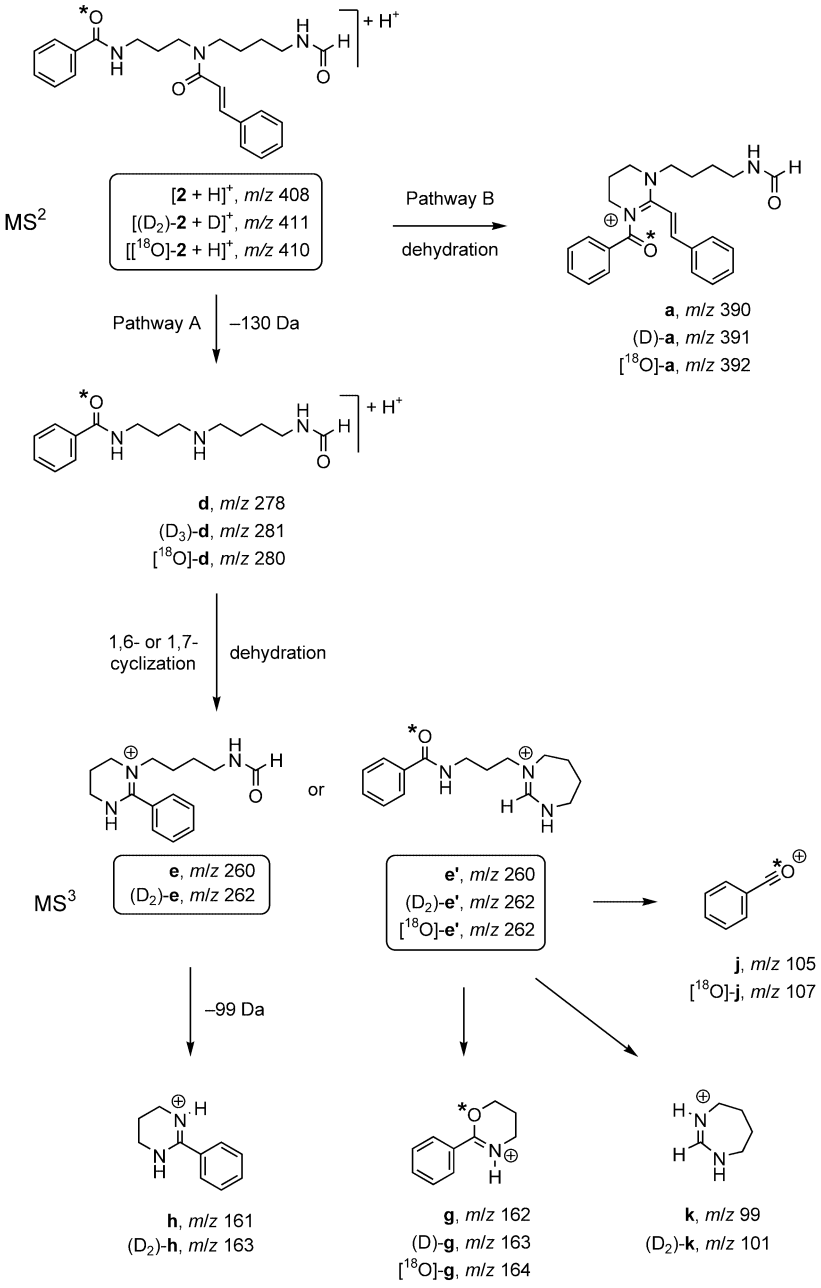


Fig. 2. *MS*^{n} *Plots of the quasi-molecular ions of chisitine 2* (**2**): a) *MS*^{2} *(408)* (black line) *and MS*^{3} *(408 → 260)* (grey line) *of* $[\mathbf{2} + H]^+$, b) *MS*^{2} *(411)* (black line) *and MS*^{3} *(411 → 262)* (grey line) *of* $[(D_2)\text{-}\mathbf{2} + D]^+$, *and* c) *MS*^{2} *(410)* (black line) *and MS*^{3} *(410 → 262)/(410 → 260)* (grey lines) *of* $[[^{18}O]\text{-}\mathbf{2} + H]^+$. Fragment ions are designated according to decreasing *m*/*z* values (**a** to **k**).

(see *Scheme 1*). The fragment ions **b** (*m*/*z* 380), **c** (*m*/*z* 286), **f** (*m*/*z* 189), and **i** (*m*/*z* 134) (*Fig. 2*,*a*) are not discussed because they are not characteristic for the structure elucidation of **2**.

Scheme 1. *MS Fragmentation of the Quasi-Molecular Ions of Chisitine 2* (**2**)[2])



[2]) The * denotes the location of the ^{18}O-atom in the case of the labeled analog [^{18}O]-**2**. The boxes indicate the selected *m*/*z* values submitted to MS^2 and MS^3. The structures of the fragment ions **b**, **c**, **f**, and **i** are not depicted.

To support the proposed mechanism, a H/D-exchange experiment was performed. Thus, **2** was dissolved in an acidic solution of MeOD (0.1% of DCl), and the resulting deuterated compound $[(D_2)\text{-}\mathbf{2}+D]^+$ (*m/z* 411) was investigated by ESI-MS and MS/MS (*Fig. 2,b*). The mass shifts observed for the fragment ions, which depend on the number of their exchangeable protons, support the proposed mechanism in *Scheme 1*. All fragment ions are summarized in *Table 3*.

Table 3. *Fragment Ions in the MS^n of Chisitine 1* (**1**) *and 2* (**2**)

	Quasi-molecular ion		MS^n Experiment	Fragment ions, *m/z* (rel. intensity)
	type	*m/z*		
1	$[\mathbf{1}+H]^+$	378	MS^2 (378)	**l**, 330(100); **m**, 302(1); **n**/**n′**, 230(1); **o**, 200(1); **p**, 182(14); **q**, 155(3)
			MS^3 (378 → 330)	**m**, 302(4); **o**, 200(6); **p**, 182(100); **q**, 155(4); **r**, 131(4)
(D_2)-**1**	$[(D_2)\text{-}\mathbf{1}+D]^+$	381	MS^2 (381)	(D_2)-**l**, 332(100); (D_2)-**m**, 304(1); (D_2)-**n**/(D_2)-**n′**, 232(2); (D_2)-**o**, 202(4); (D)-**p**, 183(14); (D)-**q**, 156(2)
			MS^3 (381 → 332)	(D_2)-**m**, 304(6); (D_2)-**o**, 202(7); (D)-**p**, 183(100); (D)-**q**, 156(1); **r**, 131(4)
2	$[\mathbf{2}+H]^+$	408	MS^2 (408)	**a**, 390(2); **b**, 380(2); **c**, 286(9); **d**, 278(6); **e**/**e′**, 260(100); **g**, 162(9); **h**, 161(6)
			MS^3 (408 → 260)	**f**, 189(9); **g**, 162(97); **h**, 161(100); **i**, 134(4); **j**, 105(14); **k**, 99(84)
(D_2)-**2**	$[(D_2)\text{-}\mathbf{2}+D]^+$	411	MS^2 (411)	(D)-**a**, 391(2); (D_3)-**b**, 383 (3); (D_2)-**c**, 288(12); (D_3)-**d**, 281(8); (D_2)-**e**/(D_2)-**e′**, 262(100); (D)-**g**/(D_2)-**h**, 163(14)
			MS^3 (411 → 262)	(D)-**f**, 190(2); (D)-**g**/(D_2)-**h**, 163(100); (D)-**i**, 135(3); **j**, 105(5); (D_2)-**k**, 101(34)
[^{18}O]-**2**	$[[^{18}O]\text{-}\mathbf{2}+H]^+$	410	MS^2 (410)	[^{18}O]-**a**, 392(3); [^{18}O]-**b**, 382(6); **c**, 286(18); [^{18}O]-**d**, 280(17); [^{18}O]-**e′**, 262(100); **e**, 260(84); [^{18}O]-**g**, 164(13); **h**, 161(7)
			MS^3 (410 → 262)	[^{18}O]-**f**, 191(10); [^{18}O]-**g**, 164(100); [^{18}O]-**i**, 136(1); [^{18}O]-**j**, 107(11); **k**, 99(89)
			MS^3 (410 → 260)	**h**, 161(100)

In the case of chisitine 1 (**1**), the presence of the (methylthio)carbonyl substituent instead of the benzoyl group of **2** has dramatic consequences on the MS behavior of **1** (*Fig. 3*). The S-containing group dominates the fragmentation paths (*Scheme 2*), which results in less information to be extracted from the MS/MS data as compared to those obtained with chisitine 2 (**2**). *Pathway A*, which begins with the cleavage of the cinnamoyl group (−130 Da) and is followed by a dehydration reaction (−18 Da) to form the two isomeric cyclic fragment ions **n** and **n′** at *m/z* 230 (in analogy to the ions **e** and **e′** in the case of **1**), is clearly unfavored (*Fig. 3* and *Scheme 2*). Despite the low intensity of the ions obtained, *Pathway A* is taking place and is attested by the formation of fragment ions **p** (*m/z* 182) when **n** and **n′** are submitted to an MS^3 experiment (spectra not shown).

Quasi-molecular ion $[\mathbf{1}+H]^+$ (*m/z* 378) does not undergo dehydration (*Pathway B*) but loses MeSH (−48 Da), following the favored *Pathway C*, to form the intense fragment ion **l** at *m/z* 330 (base peak in MS^2) (*Scheme 2*). The observation can be rationalized by the fact that MeSH is a better leaving group than H_2O. Isolation and further fragmentation of ion **l** (*m/z* 330) leads to the loss of the cinnamoyl moiety

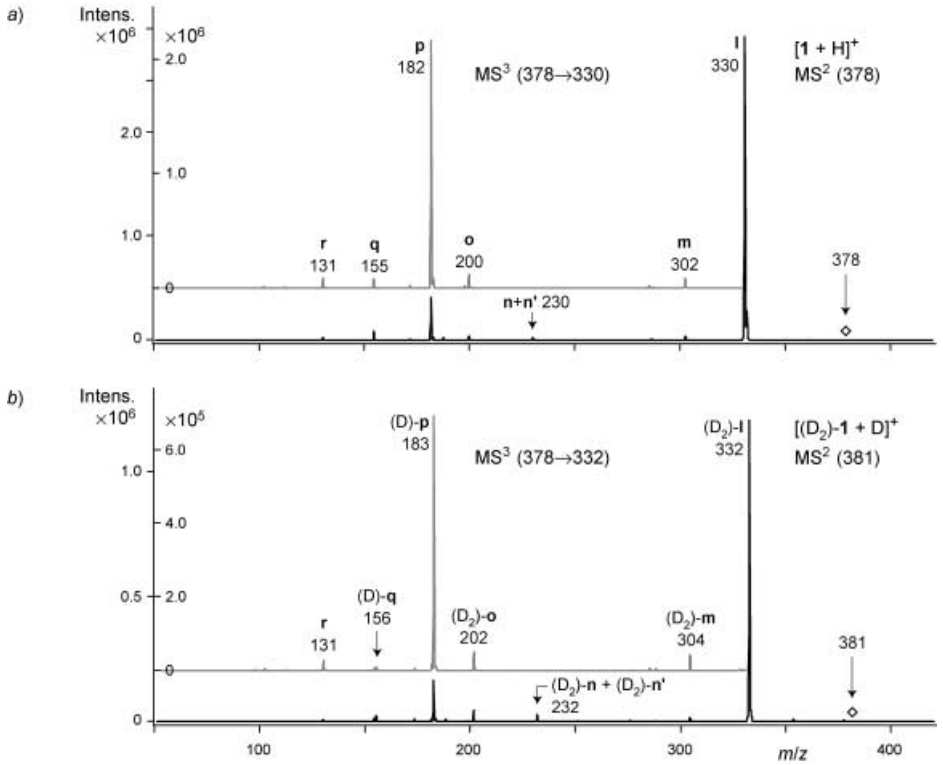


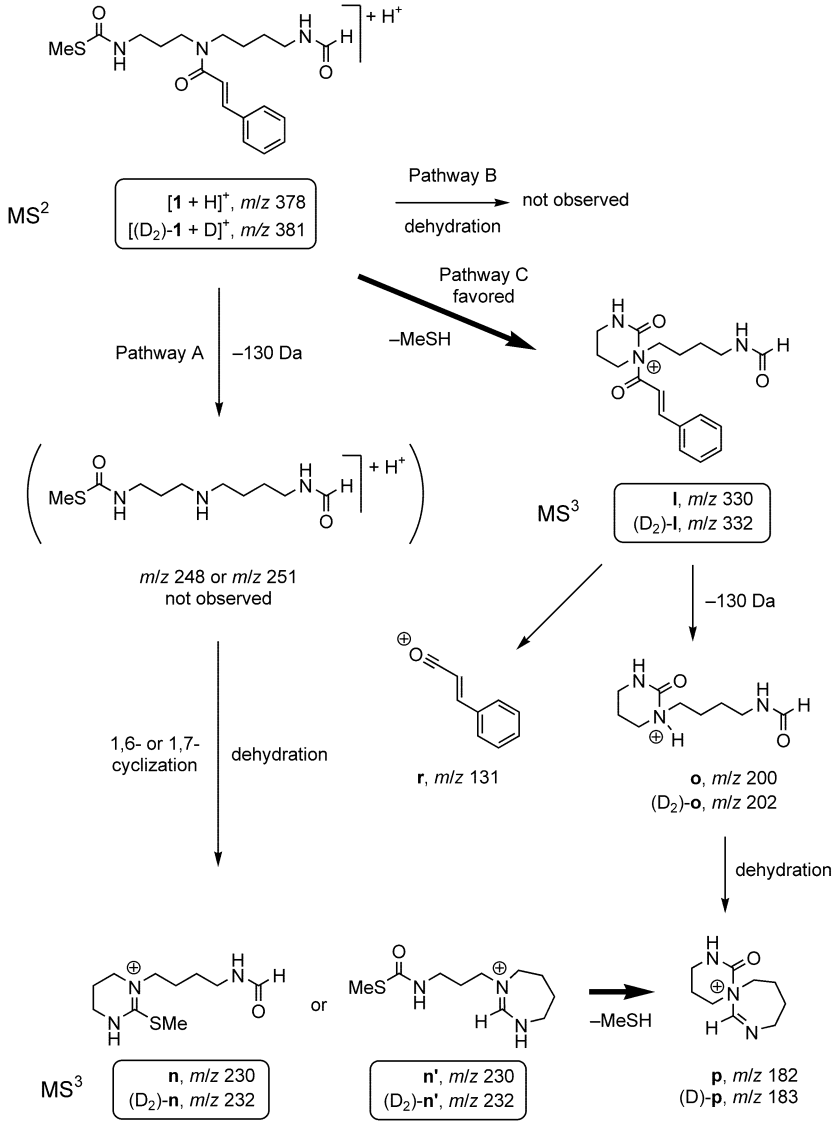
Fig. 3. *MS*^{n} *plots of the quasi-molecular ions of chisitine 1* (**1**): a) *MS*^{2} *(378)* (black line) *and MS*^{3} *(378 → 330)* (grey line) *of* $[\mathbf{1}+H]^+$, *and* b) *MS*^{2} *(381)* (black line) *and MS*^{3} *(381 → 332)* (grey line) *of* $[(D_2)\text{-}\mathbf{1}+D]^+$. Fragment ions are designated according to decreasing *m/z* value (**l** to **r**).

(−130 Da) under formation of fragment ion **o** (*m/z* 200). Subsequent dehydration gives bicyclic ion **p**, recorded at *m/z* 182 (base peak in MS^3). The latter ion can also derive from ion **n′** (*m/z* 230) by expulsion of MeSH (−48 Da). In addition, fragment ion **l** (*m/z* 330) produces the cinnamoyl cation **r** (*m/z* 131). Analogously to the above mentioned H/D-exchange experiment, compound **1** was treated in the same way to give deuterated analog $[(D_2)\text{-}\mathbf{1}+D]^+$ (*m/z* 381). The ion was investigated by ESI-MS and MS/MS (*Fig. 3,b*) to support the mechanism proposed in *Scheme 2*.

This detailed MS analysis confirmed that the structures of both alkaloids **1** and **2** were correctly assigned. Nevertheless, it seemed important to ensure them by synthesis and also to study their NMR spectra in detail.

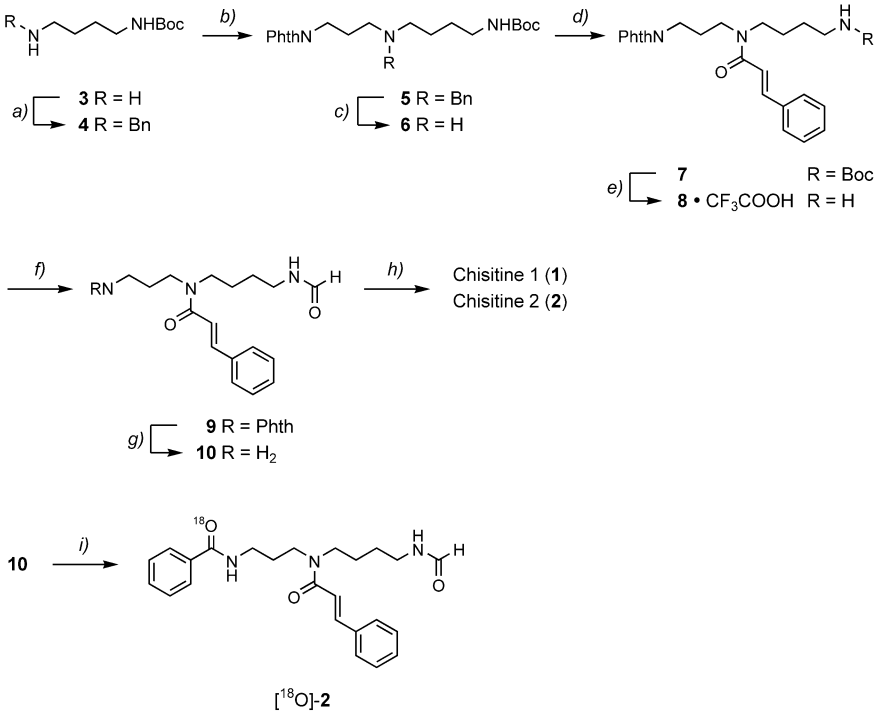
2.4. *Synthesis of Chisitine 1* (**1**) *and 2* (**2**). The two target compounds **1** and **2** were prepared starting from commercially available mono-Boc-protected butane-1,4-diamine **3**, which was benzylated by reductive amination of benzaldehyde to give intermediate **4** (*Scheme 3*). Alkylation of **4** with *N*-(3-bromopropyl)phthalimide afforded **5**, which was debenzylated to the bis-protected spermidine derivative **6** by hydrogenolysis. Subsequent derivatization of the secondary-amine function with cinnamoyl chloride (=(2*E*)-3-phenylprop-2-enoyl chloride) yielded compound **7**. The

Scheme 2. *MS Fragmentation of the Quasi-Molecular Ions of Chisitine 1* (**1**)



Boc protecting group was removed by the action of CF_3COOH, and compound **8** was obtained as the CF_3COOH salt. Formylation of **8** by treatment with 4-nitrophenyl formate gave the spermidine derivative **9**, carrying two of the desired three *N*-substituents. Introduction of the last substituent was achieved after the phthalimide-protecting group was removed by the addition of hydrazine. The resulting primary amine **10** was treated with *S*-methyl carbonochloridothioate to provide the desired alkaloid **1** in 16% overall yield. The reaction of **10** with benzoyl chloride gave

Scheme 3. *Synthetic Route to Chisitine 1* (**1**) *and 2* (**2**) *and Labeled Analog [^{18}O]-***2**



a) 1. PhCHO, MeOH, r.t., 1 h; 2. $NaBH_4$, 19 h; 95%. *b*) $Br(CH_2)_3NPhth$, K_2CO_3, 70°, 23 h; 78%. *c*) H_2 (4 bar), Pd/C, EtOH, r.t., 19 h; quant. *d*) (*E*)-$C_6H_5CH{=}CHCOCl$, Et_3N, *N*,*N*-dimethylpyridin-4-amine (DMAP), CH_2Cl_2, r.t., 6 h; 55%. *e*) CF_3COOH, CH_2Cl_2, r.t., 3 h; quant. *f*) $HCO_2(4\text{-}NO_2C_6H_4)$, Et_3N, THF, r.t., 1 h; 82%. *g*) $N_2H_4 \cdot H_2O$, EtOH, reflux, 2 h; 60%. *h*) MeSCOCl or PhCOCl, pyridine, CH_2Cl_2, r.t., 1 h; 67% (**1**) or 64% (**2**). *i*) 1. $PhC[^{18}O_2]H$, $(COCl)_2$, DMF, CH_2Cl_2, r.t., 2 h; 2. **10**, Et_3N, CH_2Cl_2, r.t., 2 h; 78%.

compound **2** in 13% overall yield. All analytical data (HPLC-MS, MS/MS, ^{1}H- and ^{13}C-NMR, as well as UV spectra) measured for the two synthetic references were identical with those of the corresponding natural alkaloids **1** and **2**. To study the fragmentation pathway during MS/MS analysis, labeled [^{18}O]-**2** was synthesized analogously by coupling **10** with ^{18}O-labeled benzoyl chloride, which was prepared according to a reported procedure [14].

3. Conclusions. – Two novel spermidine-derived triamide alkaloids were isolated from the leaves of *C. weinlandii*, chisitine 1 (**1**) and chisitine 2 (**2**). The structures of the two compounds were established on the basis of MS and NMR analysis and further confirmed by comparison with their synthetic references. It was shown that **1** and **2** are both present as a 1:1 mixture of two isomers when their NMR spectra are recorded at 300 K. The sites of attachment of the substituents were securely established by the HMBC experiment.

During the mechanistic investigation of the MS fragmentation pathways of the two compounds by ESI-MS/MS, it was observed that acyl-substituted spermidine deriva-

tives undergo dehydration by a ring-closure reaction. Another distinction comes from the dependence of the dissociation pathways on the lability of the substituents. The replacement of PhCO by a MeSCO moiety, which possesses the good leaving group MeSH, affects the MS/MS fingerprint. The MS/MS fragment ions recorded for the two related compounds arise from alternative pathways. Attempts to elucidate their structure solely on the basis of the MS/MS data are, therefore, risky.

We gratefully acknowledge the *Swiss National Science Foundation* for the generous support of this work, Ms. *N. Walch*, and the NMR service of the Institute of Organic Chemistry University of Zurich for the measurements. We also acknowledge the *Ministry of Education, Sciences, Sports, and Culture*, and the *Ministry of Health and Welfare*, Japan, for financial help.

Experimental Part

1. *General*. All chemicals were of reagent grade and purchased from *Fluka Chemie AG* or *Merck AG*. All solvents were of anal. grade and were used without further purification unless otherwise stated. THF was dried over Na/benzophenone. Hydrogenation: *Parr-Instruments Company Inc.* Flash chromatography (FC): silica gel *Merck 60* (40–63 μm, 230–400 mesh). TLC: *Merck* precoated silica-gel-*60-F_{254}* plates; detection by UV at 254 nm, or by spraying *Schlittler* reagent (H_2PtCl_6/HCl/KI) or ninhydrine reagent and heating at 300° for 1 min. IR Spectra: *Perkin-Elmer 297*; in cm^{-1}. NMR Spectra: *Bruker ARX-300* (at 300.13 (^1H) and 75.47 MHz (^{13}C)) at 300 K for all 1D ^1H, ^{13}C, DEPT-135, and DEPT-90 experiments; *DRX-600* (at 600.13 (^1H) and 150.90 MHz (^{13}C)) or *DRX-500* (at 500.13 (^1H) and 125.76 MHz (^{13}C)) at 300 K for the ^1H, ^{13}C,^1H-HSQC, ^{13}C,^1H-HMBC, ^{13}C,^1H-HSQC-TOCSY experiments of **1** and **2**; chemical shifts δ in ppm, referenced against residual non-deuterated solvent; *J* values in Hz. HPLC-MS: *Hewlett-Packard 1100* HPLC system (*Hewlett-Packard Co.*, Palo Alto, CA, U.S.A.) equipped with a *HTS-PAL* autosampler (*CTC Analytics*, Zwingen, Switzerland), connected to a *Bruker ESQUIRE-LC* quadrupole ion-trap instrument (*Bruker Daltonik GmbH,* Bremen, Germany) equipped with a combined *Hewlett-Packard* atmospheric-pressure ion (API) source (*Hewlett-Packard Co.*, Palo Alto, CA, U.S.A.). Direct-infusion ESI-MS and ESI-MS^n experiments were performed with a syringe infusion pump (*Cole-Parmer 74900-05*; *Cole-Parmer Instrument Company*, Vernon Hills, IL, U.S.A.).

2. *Isolation of Chisitine 1* (**1**) *and 2* (**2**). The leaves of *Chisocheton weinlandii* HARMS were harvested in 1993 in the garden of the Herbarium Bogoriense, Java, Indonesia, and voucher specimens have been deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Setsunan University.

The crushed leaves (60.0 g) were extracted with MeOH (4 × 1000 ml), and the solvent was distilled. The MeOH extract (10.9 g) was then suspended in H_2O (300 ml), and the aq. suspension was extracted with AcOEt (4 × 150 ml). After removal of the solvent, the extract (3.7 g) was chromatographed (silica gel, $CHCl_3$/MeOH gradient of increasing MeOH content). Fractions containing **1** and **2** (eluted with $CHCl_3$/MeOH 5:1) were further purified by semi-prep. HPLC to afford pure chisitine 1 (**1**; 26.0 mg) and chisitine 2 (**2**; 15.3 mg). For the anal. data of **1** and **2**, which are identical in all respects to those of the synthetic references, see *Sect. 4* below).

3. *HPLC-ESI-MS and ESI-MS Investigation*. Solvents and reagents: MeCN, MeOH (HPLC grade; *Scharlau*, Barcelona, Spain); CF_3COOH *purum* (*Fluka*, Buchs, Switzerland). H_2O was purified with a *Milli-Q_{RG}* apparatus (*Millipore*, Milford, MA, U.S.A). Samples preparation: 0.2 mg of solid material (natural or synthetic) was dissolved in 800 μl of MeOH. For the HPLC-ESI-MS experiments, 10 μl of the stock solns. were injected for the individual runs; for ESI-MS experiments, the solns were continuously introduced through the electrospray interface at a flow rate of 6 μl min^{-1}. For H/D-exchange experiments, 0.2 mg of solid material was dissolved in 500 μl of a 0.1% soln. of DCl in MeOD (*Merck*, Darmstadt, Germany) and stirred at 23° for 15 min prior to use. Column and chromatographic conditions: *Interchrom-Uptisphere-C_{18}-HDO* column (*UP3 HDO#20QS*, 3 μm, 2.1 × 200 mm; *Interchim*, Montluçon, France); flow rate 0.2 ml min^{-1}; mobile phase: gradient within 30 min from 20 to 50% of solvent *B* and finally within 5 min from 50 to 100% of *B* (solvent *A* = 0.1% CF_3COOH soln. in H_2O, solvent *B* = 0.1% CF_3COOH soln. in MeCN). MS Conditions: nebulizer gas (N_2) 40 psi, dry gas (N_2) 8.5 l min^{-1}, dry temp. 300°, capillary voltage 4200 V, end plate −500 V, capillary exit 86 V, and skimmer 1 18 V; data acquisitions at normal resolution (0.6 Da at half peak height), under ion charge control (ICC) conditions (10000) in the mass range from *m*/*z* 50 to 600 and an *m*/*z* 39.3 trap drive value. To get representative mass spectra, 8 scans were averaged for each spectrum.

4. *Synthesis of Chisitine 1* (**1**) *and 2* (**2**). tert-*Butyl [4-(Benzylamino)butyl]carbamate* (**4**). To a soln. of *tert*-butyl (4-aminobutyl)carbamate (**3**; 1.40 g, 7.42 mmol) in MeOH (40 ml), benzaldehyde (0.79 g, 7.43 mmol) was added and the mixture stirred at 23° for 45 min. It was cooled to 0° (ice/water), $NaBH_4$ (0.32 g, 8.46 mmol) was added portionwise within 1 h, and the soln. allowed to warm to 23° and stirred for an additional 17 h. After evaporation, the residue was dissolved in H_2O (50 ml) and extracted with CH_2Cl_2 (3 × 50 ml) and the org. phase dried ($MgSO_4$) and evaporated: **4** (1.96 g, 95%). Colorless oil. R_f (CH_2Cl_2/MeOH/25% NH_4OH 9:9:1): 0.64. IR (film): 3340*s*, 2960*s*, 2920*s*, 2850*s*, 2810*s*, 1950*w*, 1875*w*, 1810*w*, 1700*s*, 1610*m*, 1520*s*, 1455*s*, 1390*s*, 1370*s*, 1275*s*, 1250*s*, 1170*s*, 1030*s*, 920*m*, 870*m*, 780*m*, 740*s*, 700*s*. ^{1}H-NMR ($CDCl_3$): 7.31–7.17 (*m*, 5 H); 4.83 (br. *s*, 1 H); 3.73 (*s*, 2 H); 3.08–3.06 (*m*, 2 H); 2.62–2.57 (*m*, 2 H); 1.50–1.46 (*m*, 4 H); 1.39 (*s*, 9 H). ^{13}C-NMR ($CDCl_3$): 156.1 (*s*); 140.6 (*s*); 128.5 (*d*); 128.3 (*d*); 127.1 (*d*); 79.1 (*s*); 54.2 (*t*); 49.1 (*t*); 40.6 (*t*); 28.6 (*q*); 28.0 (*t*); 27.6 (*t*). ESI-MS (MeOH): 279.2 ($[M+H]^+$).

tert-*Butyl {4-{Benzyl[3-(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)propyl]amino}butyl}carbamate* (**5**). K_2CO_3 (0.52 g, 3.78 mmol) was added to a soln. of **4** (0.96 g, 3.43 mmol) in MeCN (15 ml) followed by *N*-(3-bromopropyl)phthalimide (1.01 g, 3.78 mmol), and the heterogeneous mixture was stirred at 70° for 23 h. The soln. was poured on H_2O (50 ml) and extracted with CH_2Cl_2 (3 × 50 ml), the combined org. phase dried ($MgSO_4$) and evaporated, and the crude mixture purified by FC (hexane/AcOEt 3:2 → 1:1): pure **5** (1.80 g, 78%). Slightly yellow oil. R_f (CH_2Cl_2/MeOH 1:1): 0.58. IR (film): 3360*s*, 2990*s*, 2900*s*, 2830*s*, 2780*s*, 1940*w*, 1755*s*, 1690*s*, 1600*s*, 1500*s*, 1435*s*, 1380*s*, 1230*s*, 1155*s*, 1020*s*, 900*s*, 870*s*, 855*s*, 705*s*, 685*s*, 655*s*. ^{1}H-NMR ($CDCl_3$): 7.84–7.81 (*m*, 2 H); 7.71–7.68 (*m*, 2 H); 7.31–7.17 (*m*, 5 H); 4.74 (br. *s*, 1 H); 3.69 (*t*, *J* = 7.6, 2 H); 3.52 (*s*, 2 H); 3.07–3.06 (*m*, 2 H); 2.50–2.40 (*m*, 4 H); 1.83 (*quint.*, *J* = 7.3, 2 H); 1.50–1.45 (*m*, 4 H); 1.43 (*s*, 9 H). ^{13}C-NMR ($CDCl_3$): 168.5 (*s*); 156.2 (*s*); 139.8 (*s*); 134.0 (*d*); 132.4 (*s*); 129.1 (*d*); 128.3 (*d*); 127.0 (*d*); 123.3 (*d*); 79.1 (*s*); 58.8 (*t*); 53.6 (*t*); 51.4 (*t*); 40.7 (*t*); 36.6 (*t*); 28.6 (*q*); 28.0 (*t*); 26.3 (*t*); 24.7 (*t*). ESI-MS (MeOH + NaI): 488.3 ($[M+Na]^+$).

tert-*Butyl {4-{[3-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)propyl]amino}butyl}carbamate* (**6**). A mixture of **5** (1.18 g, 2.54 mmol) and Pd/C (200 mg) in EtOH (100 ml) was stirred for 19 h at 23° under a H_2 pressure of 4 bar [15]. The catalyst was filtered off *via Celite*® and washed with MeOH (150 ml). The combined filtrate was evaporated: **6** (1.26 g, quant.). Colorless solid. R_f (CH_2Cl_2/MeOH 1:1): 0.61. IR (film): 3370*m*, 2940*m*, 2790*m*, 2440*w*, 2780*s*, 1770*w*, 1705*s*, 1690*s*, 1600*w*, 1530*m*, 1465*m*, 1440*m*, 1400*m*, 1365*m*, 1270*m*, 1250*m*, 1175*m*, 715*m*. ^{1}H-NMR ($CDCl_3$): 7.84–7.81 (*m*, 2 H); 7.72–7.69 (*m*, 2 H); 5.14 (br. *s*, 1 H); 3.85 (*t*, *J* = 6.5, 2 H); 3.17–3.11 (*m*, 2 H); 3.04–2.95 (*m*, 4 H); 2.32–2.27 (*m*, 2 H); 1.95–1.85 (*m*, 2 H); 1.66–1.47 (*m*, 2 H); 1.41 (*s*, 9 H). ^{13}C-NMR ($CDCl_3$): 168.6 (*s*); 156.5 (*s*); 134.3 (*d*); 132.1 (*s*); 123.6 (*d*); 79.5 (*s*); 47.8 (*t*); 45.8 (*t*); 39.9 (*t*); 35.2 (*t*); 28.6 (*q*); 27.3 (*t*); 25.8 (*t*); 23.3 (*t*). ESI-MS (MeOH): 320.2 (5), 376.3 (100, $[M+H]^+$), 398.2 (6, $[M+Na]^+$).

tert-*Butyl {4-{[3-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)propyl][(2*E*)-3-phenylprop-2-enoyl]amino}butyl}carbamate* (**7**). According to [16], Et_3N (3.67 g, 36.29 mmol) and DMAP (42 mg, 0.34 mmol) were added to a soln. of **6** (948 mg, 2.54 mmol) in CH_2Cl_2 (25 ml). After stirring at 23° for 30 min, (2*E*)-3-phenylprop-2-enoyl chloride (847 mg, 5.08 mmol) was added, and the mixture was stirred at 23° for an additional 6 h. The soln. was washed with sat. aq. NH_4Cl soln. (3 × 30 ml) followed by sat. aq. Na_2CO_3 soln. (3 × 30 ml). The org. layer was dried ($MgSO_4$) and evaporated. The crude mixture was purified by FC (hexane/AcOEt 1:1 → 2:3): pure **7** (707 mg, 55%). Yellow oil. R_f (hexane/AcOEt 1:1): 0.11. IR (film): 3350*m*, 3070*m*, 2980*s*, 2940*s*, 2870*m*, 1775*s*, 1710*s*, 1655*s*, 1610*s*, 1525*s*, 1475*s*, 1460*s*, 1445*s*, 1405*s*, 1375*s*, 1340*s*, 1280*s*, 1260*s*, 1180*s*, 1150*m*, 1100*m*, 1080*m*, 1045*m*, 985*m*, 900*m*, 865*m*, 775*s*, 730*s*, 695*m*, 675*m*. ^{1}H-NMR ($CDCl_3$): 7.88–7.82 (*m*, 2 H); 7.74–7.63 (*m*, 3 H); 7.52–7.43 (2 *m*, 2 H); 7.36–7.31 (*m*, 3 H); 6.83, 6.76 (2 *d*, *J* = 15.4 each, ratio 1:1, 1 H); 4.65 (br. *s*, 1 H); 3.77–3.72 (*m*, 2 H); 3.55–3.44 (*m*, 4 H); 3.18–3.12 (*m*, 2 H); 2.10–1.95 (*m*, 2 H); 1.75–1.62 (*m*, 2 H); 1.57–1.47 (*m*, 2 H); 1.41 (*s*, 9 H). ^{13}C-NMR ($CDCl_3$): 168.4 (*s*); 166.5, 166.4 (2 *s*); 156.2 (*s*); 143.0 (*d*); 135.5 (*s*); 134.3, 134.1 (2 *d*); 132.3, 132.1 (2 *s*); 129.7, 128.9, 128.0 (3 *d*); 123.6, 123.4 (2 *d*); 117.5, 117.3 (2 *d*); 79.4 (*s*); 48.0, 46.6, 46.1, 44.6 (4 *t*); 40.2 (*t*); 36.2, 35.7 (2 *t*); 29.0 (*t*); 28.6 (*q*); 27.8, 27.6, 27.4, 27.2, 25.4 (5 *t*). ESI-MS (MeOH + NaI): 528.3 ($[M+Na]^+$).

*4-{[3-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)propyl][(2*E*)-3-phenylprop-2-enoyl]amino}butan-1-ammonium Trifluoroacetate* (**8**). CF_3COOH (1.58 g, 13.85 mmol) was added dropwise to a soln. of **7** (700 mg, 1.39 mmol) in CH_2Cl_2 (10 ml), and the mixture was stirred at 23° for 3 h. Evaporation gave **8** (929 mg, quant.). Brownish oil. R_f (CH_2Cl_2/MeOH 1:1): 0.50. IR (film): 3310*m*, 1805*s*, 1745*s*, 1680*s*, 1615*m*, 1535*m*, 1505*m*, 1475*m*, 1440*s*, 1420*m*, 1370*m*, 1340*m*, 1240*s*, 1205*s*, 1110*m*, 1065*m*, 1010*m*, 850*m*, 830*m*, 805*m*, 760*s*, 745*m*. ^{1}H-NMR ($CDCl_3$): 7.74–7.62 (*m*, 4 H); 7.47 (*d*, *J* = 15.4, 1 H); 7.35–7.18 (*m*, 5 H); 6.64 (*d*, *J* = 15.4, 1 H); 3.68–3.58 (*m*, 2 H); 3.47–3.41 (*m*, 4 H); 3.12–3.10 (*m*, 2 H); 2.03–1.94, 1.92–1.87 (2 *m*, ratio 3:1, 2 H); 1.72–1.65 (*m*, 4 H). ^{13}C-NMR ($CDCl_3$): 169.5, 169.3 (2 *s*); 168.9 (*s*); 160.7 (*q*, *J*(CO,F) = 41.2); 146.0 (*d*); 134.8, 134.7 (2 *d*); 134.2 (*s*); 131.7 (2 *s*); 130.9, 129.2, 128.3 (3 *d*); 123.8, 123.7 (2 *d*); 115.2 (*q*, *J*(C,F) = 286.9); 115.3, 115.2

(2 *d*); 48.6, 47.0, 46.5, 45.8 (4 *t*); 40.5, 40.2 (2 *t*); 35.9, 35.5 (2 *t*); 28.3, 27.4, 26.4, 24.7, 24.4, 24.0 (6 *t*). ESI-MS (MeOH): 406.3 (100, $[M+H]^+$), 428.2 (6, $[M+Na]^+$).

*(2*E*)*-N-*[3-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)propyl]*-N-*[4-(formylamino)butyl]-3-phenylprop-2-enamide* (**9**). According to [17], 4-nitrophenyl formate (231 mg, 1.38 mmol) in THF (4 ml) was added dropwise to a soln. of **8** (700 mg, 1.35 mmol) and Et_3N (684 mg, 6.76 mmol) in THF (10 ml). The mixture was stirred at 23° for 1 h. Evaporation and purification of the crude product by FC (CH_2Cl_2/MeOH 93 :7 → 9 :1) gave pure **9** (481 mg, 82%). Yellowish oil. R_f (CH_2Cl_2/MeOH 9 :1): 0.39. IR (film): 3370*m*, 3140*m*, 3020*m*, 2950*m*, 1810*s*, 1750*s*, 1715*s*, 1690*s*, 1640*s*, 1575*m*, 1540*m*, 1475*s*, 1440*s*, 1420*s*, 1370*m*, 1345*m*, 1230*m*, 1175*m*, 1115*m*, 1070*m*, 1020*m*, 930*m*, 805*s*, 760*s*. ^{1}H-NMR ($CDCl_3$): 8.16 (br. *s*, 1 H); 7.86 – 7.81 (*m*, 2 H); 7.74 – 7.62 (*m*, 3 H); 7.51 – 7.31 (*m*, 5 H); 6.81, 6.76 (2 *d*, *J* = 15.4 each, ratio 1 :1, 1 H); 6.40, 6.01 (2 br. *s*, 1 H); 3.78 – 3.71 (*m*, 2 H); 3.55 – 3.43 (*m*, 4 H); 3.38 – 3.23 (*m*, 2 H); 2.10 – 1.92 (*m*, 2 H); 1.68 – 1.52 (*m*, 4 H). ^{13}C-NMR ($CDCl_3$): 168.4 (*s*); 166.6 (*s*); 164.7, 161.5 (2 *d*); 143.3, 143.1 (2 *d*); 135.4, 135.3 (2 *s*); 134.4, 134.3 (2 *d*); 132.2, 132.1 (2 *s*); 129.8, 129.0, 128.0, 123.6, 123.4 (5 *d*); 117.4, 117.1 (2 *d*); 48.1, 46.4, 46.3, 44.8 (4 *t*); 37.7 (*t*); 36.2, 35.7 (2 *t*); 29.0, 27.3, 27.2, 26.3, 25.5 (5 *t*). ESI-MS (MeOH + NaI): 456.3 ($[M+Na]^+$).

*(2*E*)*-N-*(3-Aminopropyl)*-N-*[4-(formylamino)butyl]-3-phenylprop-2-enamide* (**10**). According to [16], $N_2H_4 \cdot H_2O$ (412 mg, 8.23 mmol) was added to a soln. of **9** (360 mg, 0.83 mmol) in EtOH (10 ml), and the mixture was stirred under reflux for 2 h. After cooling, the solvent was evaporated, the residue treated with 2N aq. HCl (10 ml), the mixture filtered, and the filtrate basified with sat. aq. Na_2CO_3 soln. (15 ml). The aq. layer was extracted with CH_2Cl_2 (3 × 20 ml) and the combined org. layer dried ($MgSO_4$) and evaporated: **10** (152 mg, 60%). Yellowish oil. R_f (CH_2Cl_2/MeOH 1 :1): 0.05. IR (film): 3360*m*, 3130*m*, 3010*m*, 2940*m*, 1710*s*, 1685*s*, 1635*s*, 1535*m*, 1495*s*, 1465*s*, 1420*m*, 1365*m*, 1340*m*, 1320*m*, 1270*m*, 1240*m*, 1220*m*, 1020*m*, 805*m*, 750*m*, 725*m*. ^{1}H-NMR ($CDCl_3$): 8.14 (*s*, 1 H); 7.67 – 7.66, 7.62 – 7.60, 7.57 – 7.56 (3 *m*, ratio 43 :50 :7, 1 H); 7.51 – 7.42, 7.34 – 7.28 (2 *m*, 5 H); 6.96, 6.79, 6.41 (3 *d*, *J* ≈ 15.4 each, 1 H); 6.77, 6.54 (2 br. *s*, 1 H); 3.51 – 3.39 (*m*, 4 H); 3.34 – 3.21 (*m*, 2 H); 2.76 – 2.58 (*m*, 2 H); 1.74 – 1.50 (*m*, 8 H). ^{13}C-NMR ($CDCl_3$): 166.8, 166.7 (2 *s*); 164.8, 161.6 (2 *d*); 142.9, 142.7 (2 *d*); 135.4, 135.3 (2 *s*); 129.7, 128.9, 128.0 (3 *d*); 117.9, 117.4 (2 *d*); 47.6, 46.2, 45.8, 43.9, 39.3, 39.1, 37.7, 37.5 (8 *t*); 33.2, 31.4 (2 *t*); 27.1, 26.9, 26.4, 25.4 (4 *t*). ESI-MS (MeOH + HCOOH): 286.2 (6), 304.2 (100, $[M+H]^+$), 326.2 (6, $[M+Na]^+$).

S-*Methyl{3-{[4-(Formylamino)butyl][(2*E*)-3-phenylprop-2-enoyl]amino}propyl}carbamothioate* (= *Chisitine 1*; **1**). In analogy to [18], *S*-methyl carbonochloridothioate (43 mg, 0.39 mmol) was added dropwise to a soln. of **10** (107 mg, 0.35 mmol) and pyridine (31 mg, 0.39 mmol) in CH_2Cl_2 (3 ml) and the mixture was stirred at 23° for 1 h. Evaporation and purification of the crude mixture by FC (CH_2Cl_2/MeOH 95 :5 → 9 :1) gave pure **1** (90 mg, 67%). Yellowish oil that solidified upon standing at 4°. M.p. 89 – 90°. R_f (CH_2Cl_2/MeOH 9 :1): 0.31. IR (film): 3350*s*, 3120*m*, 3000*m*, 2940*m*, 1705*s*, 1685*s*, 1635*s*, 1570*s*, 1535*s*, 1520*s*, 1495*s*, 1470*s*, 1420*s*, 1365*m*, 1340*m*, 1255*s*, 1160*m*, 1015*m*, 800*m*, 745*m*, 725*m*. NMR: *Tables 1* and *2*. ESI-MS (MeOH): 378.2 (26, $[M+H]^+$), 400.3 (88, $[M+Na]^+$), 416.1 (100, $[M+K]^+$). MS^n: *Table 3*. HPLC-UV-MS: t_R 23.8 min; λ_{max} 204, 218, 282 nm.

N-*{3-{[4-(Formylamino)butyl][(2*E*)-3-phenylprop-2-enoyl]amino}propyl}benzamide* (= *Chisitine 2*; **2**). According to [19], benzoyl chloride (18 mg, 0.13 mmol) was added dropwise to a soln. of **10** (36 mg, 0.12 mmol) and pyridine (10 mg, 0.13 mmol) in CH_2Cl_2 (2 ml) and the mixture was stirred at 23° for 2 h. Evaporation and purification of the crude mixture by FC (CH_2Cl_2/MeOH 95 :5 → 9 :1) gave pure **2** (31 mg, 64%). Yellow oil that solidified upon standing at 4°. M.p. 102 – 103°. R_f (CH_2Cl_2/MeOH 9 :1): 0.25. IR (film): 3290*s*, 3050*m*, 2920*s*, 2860*m*, 2230*w*, 1645*s*, 1595*s*, 1535*s*, 1485*s*, 1455*s*, 1430*s*, 1380*s*, 1325*s*, 1300*s*, 1240*m*, 1190*s*, 1120*m*, 1090*m*, 1070*m*, 1025*m*, 975*m*, 910*m*, 855*m*, 800*m*, 765*s*, 700*s*. NMR: *Tables 1* and *2*. ESI-MS (MeOH): 408.3 (63, $[M+H]^+$), 430.2 (100, $[M+Na]^+$), 446.1 (70, $[M+K]^+$). MS^n: *Table 3*. HPLC-UV-MS: t_R 25.0 min; λ_{max} 220, 280 nm.

N-*{3-{[4-(Formylamino)butyl][(2*E*)-3-phenylprop-2-enoyl]amino}propyl}benz[^{18}O]amide* ([^{18}O]-**2**). $(COCl)_2$ (9 mg, 0.07 mmol) was added to a cold (ice/water) soln. of [$^{18}O_2$]benzoic acid (8 mg, 0.07 mmol; prepared according to [14]) and DMF (*ca.* 0.5 ml, catalyst) in CH_2Cl_2 (1.5 ml), and the resultant mixture was allowed to warm to 23° and stirred for an additional 2 h. After evaporation of the oil, the residue was dissolved in CH_2Cl_2 (1 ml) and the mixture cooled to 0° (ice/water) and treated with a soln. of **10** (10 mg, 0.03 mmol) and Et_3N (7 mg, 0.07 mmol) in CH_2Cl_2 (1.5 ml). After stirring at 23° for 2 h, the solvent was evaporated and the crude mixture purified by prep. TLC (CH_2Cl_2/MeOH 9 :1): [^{18}O]-**2** (11 mg, 78%). R_f (CH_2Cl_2/MeOH 9 :1): 0.25. ESI-MS (MeOH + NaI): 432.3 ($[M+Na]^+$). MS^n: *Table 3*.

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Received December 17, 2003