Inhibitory Effects on HIV-1 Protease of Tri-*p*-coumaroylspermidine from *Artemisia caruifolia* and Related Amides

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From a methanol extract of *Artemisia caruifolia*, which showed a moderate inhibitory activity on HIV-1 protease in a preliminary screening, N^1,N^5,N^{10}-tri-*p*-coumaroylspermidine and three dicaffeoylquinic acids were isolated. The former compound was found to appreciably inhibit HIV-1 protease. Of related amides which were chemically synthesized, N^1,N^5,N^{10},N^{14}-tetra-*p*-coumaroylspermine and N^1,N^4,N^7,N^{10},N^{13}-penta-*p*-coumaroyltetraethylenepentamine inhibited HIV-1 protease more potently than N^1,N^5,N^{10}-tri-*p*-coumaroylspermidine.

Key words *Artemisia caruifolia*; tri-*p*-coumaroylspermidine; tetra-*p*-coumaroylspermine; penta-*p*-coumaroyltetraethylenepentamine; dicaffeoylquinic acid; human immunodeficiency virus type 1 protease inhibitor

During the replication of AIDS virus (HIV), the viral polyprotein must be cleaved by viral protease (PR) to generate essential viral enzymes, such as reverse transcriptase, integrase and PR itself, as well as the viral structural proteins. HIV PR has been proved to be one of the therapeutic targets and some peptide mimetic compounds targeting the active site of HIV PR have been synthesized as potent anti-HIV drugs able to be used clinically. However, high dosages of these drugs are usually needed, which has led to severe side effects and thus long term administration is difficult for AIDS patients to adhere to.[1)] This means that novel types of anti-HIV agents are urgently needed.

In the present paper, we report the isolation of N^1,N^5,N^{10}-tri-*p*-coumaroylspermidine as an HIV-1 PR inhibitor from *Artemisia caruifolia* and the synthesis and inhibitory activity of some related amides.

Results and Discussion

In a preliminary screening, a methanol extract of *Artemisia caruifolia* was found to have a moderate inhibitory activity on HIV-1 PR (38.5% at 100 μg/ml). This extract was divided into $CHCl_3$-, AcOEt-, BuOH- and H_2O-soluble fractions. Improved inhibitory activity was found in the $CHCl_3$- and AcOEt-soluble fractions, which showed 44.6% and 44.9% inhibition, respectively, at the same concentration. The BuOH- and H_2O-soluble fractions showed no activity.

The $CHCl_3$-soluble fraction was further separated into several sub-fractions, and from these, a guaianolide,[2)] a germacranolide,[2)] eight guaiane dimers,[2)] two triterpenes,[3)] a flavonoid,[3)] and eight lignans[3)] were isolated. These compounds showed inhibitory activity on HIV-1 protease of 22—46% at 100 μg/ml.

The AcOEt soluble part was further divided into 3 fractions by octadecylsilyl (ODS) column chromatography (40% MeOH eluate, 65% MeOH eluate and MeOH eluate). Increased inhibitory activity was observed in the second eluate (53.9% at 100 μg/ml) while the other two eluates showed only weak activity (21—25% inhibition at 100 μg/ml). Repeated chromatography of the 65% MeOH eluate on silica gel with $CHCl_3$–MeOH (8 : 2) and Sephadex LH-20 with 20—100% MeOH afforded compounds **1**—**4**.

Compounds **2**—**4** were identified as methyl 3,5-dicaffeoylquinate,[4)] 1,3-dicaffeoylquinic acid[5)] and 3,5-dicaffeoylquinic acid[6)] by comparing their spectral data with those reported. The structure elucidation of compound **1** was performed as follows.

The molecular weight of **1** was determined to be 583 by electrospray ionization (ESI)-MS. In its ^{1}H-NMR spectrum, compound **1** displayed signals of large coupling constants (J=15.5—16.0 Hz) at δ 6.35—6.45 and 7.55—7.60, characteristic of the spinning system of the olefinic part of *E*-*p*-coumaroyl. The signals of aromatic protons were greatly overlapped. The ^{13}C-NMR spectrum showed signals attributable to *E*-*p*-coumaroyl moieties. The signals at the upper field could be assigned to a spermidine moiety by analysis of its two dimensional (2D) NMR spectrum. The structure of **1** was then deduced to be N^1,N^5,N^{10}-tri-*p*-coumaroylspermidine. This compound had been reported from the flowers of *Crataegus* species as a mixture of *E* and *Z* forms. The structure had been established by spectral analysis of its permethylated product.[7)] Most recently, this compound was isolated from the species of the genera *Aphelandra*[8)] and *Helianthus annuus* L.[9)] However, the ^{1}H-NMR spectrum of this compound had not yet been assigned clearly. In the present experiment, this compound was isolated as a pure *E*-form and its 500 MHz ^{1}H-NMR spectrum could be interpreted by detailed analysis of its 2D NMR spectra. Though the spectrum was considerably complex due to the overlapping and splitting of signals, it revealed that all of the three *p*-coumaroyl residues were in *E*-forms and only two conformational isomers were present. As represented by proton signals of 7, 8, 7′, 8′, 7″ and 8″, the nearer a proton to the center nitrogen, the larger was the split of its signal. This phenomenon indicated that the splitting of signals was mainly due to the sterically hindered umbrella-like inversion at the center N atom. The structure of **1** was further confirmed by synthesis to be N^1,N^5,N^{10}-tri-*p*-coumaroylspermidine.

Compounds **1**—**4** were tested for their inhibitory activity on HIV-1 PR. In comparing their potent inhibitory activity on HIV-1 integrase and moderate anti-HIV activity,[10,11)] **2**—**4** showed no inhibitory activity against HIV-1 PR. Appreciable HIV-1 PR inhibitory activity was observed only in **1**. In addition, the corresponding amine (spermidine) and acid (*E*-*p*-coumaric acid) showed no activity against HIV-1 PR. To develop more potent inhibitors, several similar amides were synthesized and tested for their inhibitory activity against HIV-1 PR. Two of the synthesized amides (**7**, **8**) with longer chains, more amide bonds and free hydroxy groups in their

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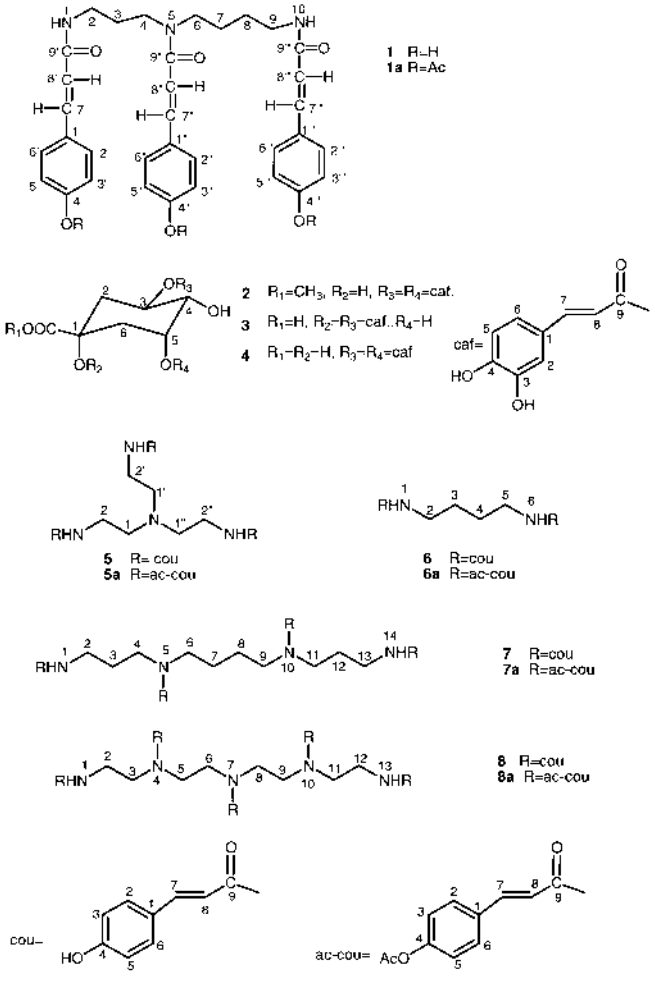
Table 1. Inhibitory Activity of Compounds Isolated from the EtOAc Extract of *A. caruifolia* and Related Amides on HIV PR

Compound	% Inhibition (100 μg/ml)	IC_{50} (μg/ml)
1	70.2±1.4	53
2	30.1±7.8	>100
3	0.0±3.2	>100
4	2.5±1.4	>100
5	2.7±0.6	>100
6	4.0±4.3	>100
7	99.5±0.8	27
8	97.3±1.7	30



p-coumaroyl moieties were found to be more potent HIV-1 PR inhibitors than **1**. However, tris(2-*p*-coumaroylaminoethyl)amine (**5**), which possesses the same number of amide bonds as **1**, showed no inhibitory activity on HIV-1 PR. On acetylation of hydroxyl groups of the *p*-coumaroyl residues as in **1a**, **7a** and **8a**, the activity disappeared completely. These polyamides are a new type of HIV-1 PR inhibitors and are easy to synthesize from inexpensive starting materials, which may enable investigaton of their interaction with the enzyme at the molecular level, and conduct of the rational design and synthesis of more preferable inhibitors against HIV-1 PR.

Experimental

Apparatus ^{1}H- and ^{13}C-NMR spectra were measured with a Varian GEMINI 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) or Varian UNITY 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) or JEOL JNM-LA 400WB-FT (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer, the chemical shifts being represented as ppm with tetramethylsilane as an internal standard. ESI-MS spectra were measured with a Perkin-Elmer SCIEX API-III biomolecular mass analyzer. FAB-MS and high resolution (HR)-FAB-MS were measured with a JEOL JMS-700T mass spectrometer, and *m*-nitrobenzyl alcohol was used as a matrix.

Plant Material The aerial part of *Artemisia caruifolia* Buch.-Ham. *ex* Roxb. was purchased from Yaocaigongyingzhan of Huhhot, Inner Mongolia of the People's Republic of China in September of 1998. The plant material was identified by Dr. Katsuko Komatsu of the Analytical Research Center for Ethnomedicines, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University. A voucher specimen (TMPW No. 19154) is stored at the Museum of Materia Medica, Toyama Medical and Pharmaceutical University, Japan.

Extraction and Isolation The aerial part of *A. caruifolia* (3.0 kg) was extracted with MeOH under reflux (20 l×3, each 2 h). The combined MeOH solutions were evaporated to give a residue (190 g). The residue was suspended in water and extracted with $CHCl_3$, AcOEt, and BuOH to give the respective fractions in yields of 96, 11 and 21 g, together with the residual water-soluble fraction (62 g). The AcOEt-soluble fraction was chromatographed on an ODS column eluted with increasing amounts of MeOH in H_2O (40% MeOH eluate, 3.4 g; 65% MeOH eluate, 5.1 g; MeOH eluate, 1.1 g). Further repeated chromatography of the second eluate on silica gel with $CHCl_3$–MeOH (8 : 2) and Sephadex LH-20 with 20—100% MeOH afforded compounds **1**—**4** (30, 40, 30 and 50 mg, respectively).

N^1,N^5,N^{10}-Tri-*p*-coumaroylspermidine (**1**): White powder, IR (KBr): ν_{max} 3300 (OH), 1660 (CONH–), 1610, 1580, 1520, 1450 (benzene ring), 1220, 1160, 980, 830, 520 cm^{-1}. Negative ESI-MS: *m/z* 582 ([M−1]$^-$, 70), 462 (50), 342 (45), 205 (30), 115 (100). Positive ESI-MS: *m/z* 607 ([M+Na]$^+$, 30), 584 ([M+H]$^+$, 35), 438 (70), 204 (70), 147 (100). ^{1}H-NMR (500 MHz, CD_3OD): δ: 1.60 (2H, m, H-8), 1.67 (2H, m, H-7), 1.85 (quin. J=7.0 Hz)/1.92 (quin. J=7.0 Hz) (2H, H-3), 3.33 (4H, m, H-2, 9), 3.52 (4H, m, H-4, 6), 6.37 (d, J=15.0 Hz)/6.40 (d, J=15.0 Hz) (H-8′), 6.42 (d, J=15.5 Hz) (H-8‴), 6.71 (d, J=9.0 Hz)/6.76 (overlapped) (H-3″, 5″), 6.77 (overlapped)/6.78 (overlapped) (H-3′, 5′), 6.79 (overlapped) (H-3‴, 5‴), 6.81 (d, J=15.5 Hz)/6.86 (d, J=15.5 Hz) (H-8″), 7.35 (d, J=9.0 Hz)/7.39 (d, J=9.0 Hz) (H-2′, 6′), 7.39 (d, J=9.0 Hz) (H-2‴, 6‴), 7.43 (overlapped)/7.44 (overlapped) (H-7′), 7.43 (overlapped)/7.47 (d, J=9.0 Hz) (H-2″, 6″), 7.47 (d, J=15.5 Hz)/7.48 (d, J=15.5 Hz) (H-7‴), 7.50 (d, J=15.5 Hz)/7.53 (d, J=15.5 Hz) (H-7″). ^{13}C-NMR (100 MHz, CD_3OD): δ: 26.3/27.9 (C-7), 27.8/27.9 (C-8), 28.9/30.6 (C-3), 37.9/38.2 (C-2), 39.9/40.1 (C-9), 45.7/47.0 (C-4), 47.6/49.0 (C-6), 114.9 (C-8″), 116.8 (C-3′, 3″, 3‴, 5′, 5″, 5‴), 118.2/118.3 (C-8′), 118.5 (C-8‴), 127.7 (C-1′, 1‴), 127.9 (C-1″), 130.6 (C-2′, 6′, 2‴, 6‴), 130.9 (C-2″, 6″), 141.8/141.9/142.2 (C-7′, 7‴), 144.4 (C-7″), 160.5/160.7 (C-4′, 4″, 4‴), 169.3 (C-9′), 169.2/169.4 (C-9″), 169.3 (C-9‴).

Methy 3, 5-Dicaffeoylquinate (**2**)[4]: White powder, positive ESI-MS *m/z* 553 ([M+Na]$^+$, 100), 385 (40). Negative ESI-MS *m/z*: 529 ([M−1]$^-$, 100), 367 (80), 179 (77). ^{1}H-NMR (500 MHz, CD_3OD): δ: 2.14 (dd, J=13.5, 8.5 Hz, Ha-2), 2.18 (dd, J=13.5, 3.5 Hz, Ha-6), 2.29 (dd, J=13.5, 6.5 Hz, Hb-6), 2.32 (dd, J=13.5, 4.0 Hz, Hb-2), 3.68 (3H, –OCH_3), 3.98 (dd, J=6.5, 3.0 Hz, H-4), 5.31 (m, H-5), 5.39 (dt, J=8.5, 4.0 Hz, H-3), 6.21 (d, J=16.0 Hz, H-8″), 6.34 (d, J=16.0 Hz, H-8′), 6.77* (d, J=8.5 Hz, H-5′), 6.78* (d, J=8.5 Hz, H-5″), 6.95** (dd, J=8.5, 2.5 Hz, H-6″), 6.96** (dd, J=8.5, 2.5 Hz, H-6′), 7.05 (d, J=2.5 Hz, H-2′), 7.06 (d, J=2.5 Hz, H-2″), 7.54 (d, J=16.0 Hz, H-7′), 7.61 (d, J=16.0 Hz, H-7″). (*, **: Assignments may be exchangeable.)

1,3-Dicaffeoylquinic Acid (**3**)[5]: White powder, positive ESI-MS *m/z* 539 ([M+Na]$^+$, 100), 413 (80), 385 (50). ^{1}H-NMR (400 MHz, CD_3OD): δ: 2.02 (dd, J=13.9, 8.9 Hz, Ha-2), 2.38 (2H, m, H-6), 2.52 (dd, J=13.9, 3.8 Hz, Hb-2), 3.74 (dd, J=8.3, 3.4 Hz, H-4), 4.25 (q, J=4.3 Hz, H-5), 5.34 (td, J=8.3, 3.6 Hz, H-3), 6.25* (d, J=16.3 Hz, H-8′), 6.26* (d, J=16.0 Hz, H-8″), 6.74 (2H, d, J=8.3 Hz, H-5′, 5″), 6.92 (2H, dd, J=8.3, 2.0 Hz, H-6′, 6″), 7.01 (2H, d, J=2.0 Hz, H-2′, 2″), 7.54 (2H, d, J=16.3 Hz, H-7′, 7″). (*: Assignments may be exchangeable.)

3,5-Dicaffeoylquinic Acid (**4**)[6]: White powder, positive ESI-MS *m/z* 539 ([M+Na]$^+$, 100), 413 (70). ^{1}H-NMR (300 MHz, CD_3OD): δ: 2.26 (4H, m, H-2, 6), 4.01 (dd, J=7.5, 3.3 Hz, H-4), 5.46 (2H, m, H-3, 5), 6.30* (d, J=16.0 Hz, H-8′), 6.39* (d, J=16.0 Hz, H-8″), 6.82 (2H, d, J=8.1 Hz, H-5′, 5″), 7.01 (2H, dd, J=8.1, 1.5 Hz, H-6′, 6″), 7.10 (2H, d, J=1.5 Hz, H-2′, 2″), 7.62** (d, J=16.0 Hz, H-7′), 7.66** (d, J=16.0 Hz, H-7″). (*, **: Assignments may be exchangeable.)

Synthesis of Polycoumaroylamines A mixture of *E*-*p*-coumaric acid (1.0 g) and Ac_2O (10 ml) in 10 ml of pyridine was stirred over night at room

temperature and worked up as usual to afford 1.1 g of (*E*)-4-*O*-acetylcoumaric acid. One gram of (*E*)-4-*O*-acetylcoumaric acid in 7 ml of $SOCl_2$ was refluxed at 80 °C for 16 h, then the mixture was evaporated to dryness to give (*E*)-4-*O*-acetylcoumaroyl chloride.

To (*E*)-4-*O*-acetylcoumaroyl chloride (0.5 mmol) in 15 ml tetrahydrofuran (THF) was added 15 ml THF solution of 0.17 mmol of spermidine [or 0.17 mmol of tris (2-aminoethyl)amine, or 0.13 mmol of spermine, or 0.25 mmol of putrescine, or 0.10 mmol of tetraethylenepentamine], and 100 μl triethylamine. The mixture was stirred at room temperature overnight and then water (20 ml) was added. After being concentrated, the residue was purified with ODS column chromatography eluted with H_2O–MeOH (6 : 4—0 : 10) to afford the corresponding 4-*O*-acetylcoumaroylamides (**1a**, **5a**—**8a**). To a solution of 4-*O*-acetylcoumaroylamide (50 mg) in CH_3OH–THF (1 : 1) was added 0.1 N KOH/MeOH 12 ml. After stirring for 1.5 h at room temperature, the solution was neutralized with 0.1 N HCl and purified by ODS column chromatography eluted with H_2O–MeOH (8 : 2—0 : 10) to give the corresponding coumaroylamides (**1**, **5**—**8**).

N^1,N^5,N^{10}-Tri(4-*O*-acetylcoumaroyl)spermidine (**1a**): White powder, 69% yield, positive FAB-MS: *m/z* 710 ($[M+1]^+$, 15). ^{1}H-NMR (300 MHz, CD_3OD): δ: 1.68 (4H, m, H-7, 8), 1.89 (2H, m, H-3), 2.25 (9H, s, CH_3CO–), 3.31 (4H, overlapped, H-2, 9), 3.58 (4H, m, H-4, 6), 6.52 (d, J=16.0 Hz)/6.57 (d, J=16.0 Hz), 6.57 (1H, d, J=16.0 Hz), 6.97 (2H, m), 7.11 (5H, m), 7.58 (9H, m) (aromatic and olefinic protons). HR-FAB-MS *m/z* 710.3086 (Calcd for $C_{40}H_{44}N_3O_9$ $[M+H]^+$, 710.3078).

Tris[2-(4-*O*-acetylcoumaroyl)aminoethyl]amine (**5a**): White powder, 53% yield, positive FAB-MS: *m/z* 711 ($[M+1]^+$, 30), 492 (10). ^{1}H-NMR (300 MHz, CD_3OD): δ: 2.29 (9H, s, CH_3CO–), 2.70 (6H, m, H-1, 1′, 1″), 3.43 (6H, m, H-2, 2′, 2″), 6.63 (3H, d, J=15.8 Hz, H-ac-cou-8), 6.86 (6H, d, J=8.4 Hz, H-ac-cou-3, 5), 7.25 (6H, d, J=8.4 Hz, H-ac-cou-2, 6), 7.39 (3H, d, J=15.8 Hz, H-ac-cou-7). HR-FAB-MS *m/z* 711.3002 (Calcd for $C_{39}H_{43}N_4O_9$ $[M+H]^+$, 711.3031).

Tris(2-*p*-coumaroylaminoethyl)amine (**5**): White powder, 89% yield, positive FAB-MS: *m/z* 585 ($[M+1]^+$, 25), 307 (15). ^{1}H-NMR (300 MHz, CD_3OD): δ: 2.67 (6H, t, J=5.1 Hz, H-1, 1′, 1″), 3.39 (6H, t, J=5.1 Hz, H-2, 2′, 2″), 6.49 (3H, d, J=15.7 Hz, H-cou-8), 6.59 (6H, d, J=8.5 Hz, H-cou-3, 6), 7.19 (6H, d, J=8.5 Hz, H-cou-2, 6), 7.39 (3H, d, J=15.7 Hz, H-cou-7). HR-FAB-MS *m/z* 585.2714 (Calcd for $C_{33}H_{37}N_4O_6$ $[M+H]^+$, 585.2714).

N^1,N^6-Di(4-*O*-acetylcoumaroyl)putrescine (**6a**): White powder, 64% yield, positive FAB-MS: *m/z* 465 ($[M+1]^+$, 15), 307 (10), 289 (8). ^{1}H-NMR (300 MHz, $CDCl_3$–CD_3OD 1 : 2): δ: 1.64 (4H, m, H-3, 4), 2.32 (6H, s, CH_3CO–), 3.36 (4H, overlapped with solvent, H-2, 5), 6.49 (2H, d, J=15.0 Hz, H-ac-cou-8), 7.11 (4H, d, J=8.5 Hz, H-ac-cou-3, 5), 7.53 (2H, d, J=15.0 Hz, H-ac-cou-7), 7.55 (4H, d, J=8.5 Hz, H-ac-cou-2, 6). HR-FAB-MS *m/z* 465.2016 (Calcd for $C_{26}H_{29}N_2O_6$ $[M+H]^+$, 465.2026).

N^1,N^6-Di-*p*-coumaroylputrescine (**6**)[12]: White powder, 90% yield, no peak appeared in ESI-MS, ^{1}H-NMR (300 MHz, CD_3OD): δ: 1.64 (4H, m, H-3, 4), 3.36 (4H, overlapped with solvent, H-2, 5), 6.43 (2H, d, J=16.0 Hz, H-cou-8), 6.82 (4H, d, J=8.6 Hz, H-cou-3, 5), 7.43 (4H, d, J=8.6 Hz, H-cou-2, 6), 7.48 (4H, d, J=16.0 Hz, H-cou-7).

N^1,N^5,N^{10},N^{14}-Tetra(4-*O*-acetylcoumaroyl)spermine (**7a**)[13]: White powder, 70% yield, positive ESI-MS: *m/z* 978 ($[M+Na]^+$, 100), 955 ($[M+H]^+$, 75). negative ESI-MS: *m/z* 953 ($[M-H]^-$, 7). ^{1}H-NMR (300 MHz, $CDCl_3$–CD_3OD 2 : 1): δ: 1.68 (4H, m, H-7, 8), 1.84 (4H, m, H-3, 12), 2.31 (12H, s, CH_3CO–), 3.42 (4H, overlapped with solvent, H-2, 13), 3.51 (8H, m, H-4, 6, 9, 11), 6.48 (2H, m), 6.81 (2H, m), 7.08 (8H, m), 7.55 (12H, m) (H-ac-cou).

N^1,N^5,N^{10},N^{14}-Tetra-*p*-coumaroylspermine (**7**)[13]: White powder, 91% yield, negative ESI-MS: *m/z* 785 ($[M-H]^-$, 7). ^{1}H-NMR (300 MHz, CD_3OD): δ: 1.69 (4H, m, H-7, 8), 1.86 (4H, m, H-3, 12), 3.31 (4H, overlapped with solvent, H-2, 13), 3.54 (8H, m, H-4, 6, 9, 11), 6.40 (2H, m), 6.79 (10H, m), 7.40 (12H, m) (H-cou).

N^1,N^4,N^7,N^{10},N^{13}-Penta(4-*O*-acetylcoumaroyl)tetraethylenepentamine (**8a**): White powder, 51% yield, positive FAB-MS: *m/z* 1130 ($[M+1]^+$, 25), 307 (13). ^{1}H-NMR (300 MHz, $CDCl_3$): δ: 2.30 (15H, s, CH_3CO–), 3.5—3.8 (16H, m, H-2, 3, 5, 6, 8, 9, 11, 12), 6.3—6.5 (2H, m), 6.9—7.7 (28H, m) (H-ac-cou). HR-FAB-MS *m/z* 1130.4347 (Calcd for $C_{63}H_{64}N_5O_{15}$ $[M+H]^+$, 1130.4400).

N^1,N^4,N^7,N^{10},N^{13}-Penta-*p*-coumaroyltetraethylenepentamine (**8**): White powder, 87% yield, positive FAB-MS: *m/z* 920 ($[M+1]^+$, 7), 307 (15), 289 (10). ^{1}H-NMR (300 MHz, CD_3OD): δ: 3.4—3.9 (16H, m, H-2, 3, 5, 6, 8, 9, 11, 12), 6.2—6.5 (2H, m), 6.6—7.0 (13H, m), 7.2—7.6 (15H, m) (H-ac-cou). HR-FAB-MS *m/z* 920.3834 (Calcd for $C_{53}H_{54}N_5O_{10}$ $[M+H]^+$, 920.3872).

HIV PR Assay HIV PR assay kit (3700 Horizon Drive, King of Prussia, PA 19406, Kit Lot No 1) was used. Assay was performed and inhibitory activity was calculated as described previously.[14] Acetyl pepstatin was used as a positive control and showed an IC_{50} of 0.07 μM.

References

1) Molla A., Granneman G. R., Sun E., Kempf D. J., *Antiviral Res*., **39**, 1—23 (1998).
2) Ma C-M., Nakamura N., Hattori M., Zhu S., Komatsu K., *J. Nat. Prod*., **63**, 1626—1629 (2000).
3) Ma C-M., Nakamura N., Min B. S., Hattori M., *Chem. Pharm. Bull*., **49**, 183—187 (2001).
4) Lin L. C., Kou Y. C., Chou C. J., *J. Nat. Prod*., **62**, 405—408 (1999).
5) Clifford M. N., *Phytochemistry*, **25**, 1767—1769 (1986).
6) Timmermann B. N., Hoffmann J. J., *J. Nat. Prod*., **46**, 365—368 (1983).
7) Strack D., Eilert U., Wray V., Wolff J., Jaggy H., *Phytochemistry*, **29**, 2893—2896 (1990).
8) Werner C., Hu W., Lorenzi-Riatsch A., Hesse M., *Phytochemistry*, **40**, 461—465 (1995).
9) Lin S., Mullin C. A., *J. Agric. Food Chem*., **47**, 1223—1229 (1999).
10) Robinson W. E. Jr., Reinecke M. G., Abdel-Malek S., Jia Q., Chow S. A., *Proc. Natl. Acad. Sci. U.S.A*., **93**, 6326—6331 (1996).
11) Robinson W. E. Jr., Cordeiro M., Abdel-Malek S., Jia Q., Chow S. A., Reinecke M.G., Mtchell W. M., *Mol. Pharmacol*., **50**, 846—855 (1996).
12) Martin-Tanguy J., Cabanne F., Perdrizet E., Martin C., *Phytochemistry*, **17**, 1927—1928 (1978).
13) Yamamoto A., Nakamura K., Otsuka M., Eur. Pat. Appl. EP 1018506 A1 12 Jul 2000, 11 pp.
14) Ma C-M., Nakamura N., Miyashiro H., Hattori M., Shimotohono K., *Chem. Pharm. Bull*., **47**, 141—145 (1999).