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Solid-Phase Synthesis of Diamine and Polyamine Amino Acid Derivatives as HIV-1 Tat-TAR Binding Inhibitors

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Abstract—A series of diamine and polyamine derivatives, either free amines or salts (HCl or TFA), of aspartic and glutamic acid were prepared in excellent yields using Rink Amide solid-phase synthesis. The asparagine and glutamine derivatives were all evaluated for their ability to inhibit Tat-TAR binding using a FIGS cellular assay, with the polyamine derivatives exhibiting the most promising binding activity.

Introduction

Approaches to the treatment of AIDS concentrate on the differences between the human immunodeficency virus (HIV) and human biochemistry. HIV utilises the host's own cell machinery to replicate limiting the differences in mammalian and viral biochemistry which can be exploited for antiviral therapy.[1,2] One important difference that can be exploited for anti-HIV drug therapy is in the processing of mRNA by the virus. Once the HIV provirus is integrated into the host DNA, it is subject to transcriptional control by cellular DNA binding proteins. Transcription of the HIV genome is regulated by virally encoded regulatory proteins, *tat* and *rev*, which produce their effect by binding to specific regions of the viral RNA during transcription.[3,4] Tat protein binds to a sequence of virally-encoded RNA called the trans activation responsive region (TAR),[5] which is found at the start of the proviral RNA, while rev binds to the rev response element (RRE).[6] Following binding of Tat to TAR transcription of viral RNA increases by 1000-fold.[7] Therefore transcriptional control on the viral-cycle of human immunodeficiency syndrome (HIV) by viral enzymes tat and rev is of considerable interest.

Tat contains arginine rich regions, which participate in RNA binding by forming two hydrogen bonds with a guanosine unit (G26) of TAR and electrostatic contact with two phosphate units in TAR (P22 and P23).[8] Targeting of tat-TAR binding has been shown to be possible by an arginamide amino acid derivative, which competes with tat for the TAR binding site resulting in a reduction of the tat–TAR binding.[4] Tat–TAR binding inhibitors require amino functional groups which can mimic the arginine rich region of tat and competitively inhibit tat–TAR binding.

This report describes the solid-phase synthesis of a series of diamine and polyamine derivatives of aspartic and glutamic acid. Interaction of polyamines with DNA molecules has been shown to result in stabilisation of their conformation through the electrostatic attraction between polycationic polyamine molecules and negatively charged phosphate groups on the DNA molecules.[9,10] Therefore diamine and polycationic polyamine derivatvies of asparagine and glutamine, were expected to bind strongly with the phosphate groups on the TAR RNA molecule resulting in tat–TAR binding inhibition. Using different amine chain lengths and number of amino/polycationic groups within the carbon chains, the optimal length and number of cationic sites required for tat–TAR binding inhibitory activity was studied.

Chemistry

The synthesis of the diamine derivatives of the amino acids, aspartic and glutamic acid, was carried out using

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solid phase synthesis on Rink amide MBHA resin,[11] commencing from Fmoc-aspartic-acid-α-allyl ester (**1**) and Fmoc-glutamic-acid-α-allyl ester (**2**) respectively (Scheme 1). After each step of the solid-phase synthesis extensive washings with DMF, H_2O, CH_2Cl_2 and MeOH were performed to remove any excess reagent and soluble by-products. **1** and **2** were coupled to the Rink amide resin via its side chain, using an excess of the activating reagent *O*-benzothiazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU)[12] and diisopropylethylamine (DIPEA) (100% loading assumed). The resulting resin-linked compounds **R3** and **R4** were treated with 20% piperidine in DMA to remove the Fmoc protecting group, then benzoic acid coupled to the resulting free amine after activation with TBTU/1-hydroxybenzotriazole (HOBt). The *O*-allyl protecting group was then deprotected using $Pd(PPh_3)_4$ and a degassed solution of CH_2Cl_2/AcOH/*N*-methyl morpholine,[13] to give the free acid **R5** or **R6**.

R5 was divided into four portions and each reacted with a different Boc-protected diamine, using TBTU/HOBt to activate the carboxylic acid, resulting in coupling of the amines at the C-terminal residue. Cleavage of the diamine asparagine derivatives, **R7a–d**, from the resin was achieved using 10% trifluoroacetic acid (TFA) in CH_2Cl_2 with 1% triisopropylsilane (TIS) as a cation scavenger. The diamine derivatives of asparagine **9a–d** were obtained in excellent yields either as the free base, HCl or TFA salt (Table 1), depending on the work up procedure (see Experimental). In a similar manner, the diamine derivatives of glutamine **10a–d** were obtained (Scheme 1).

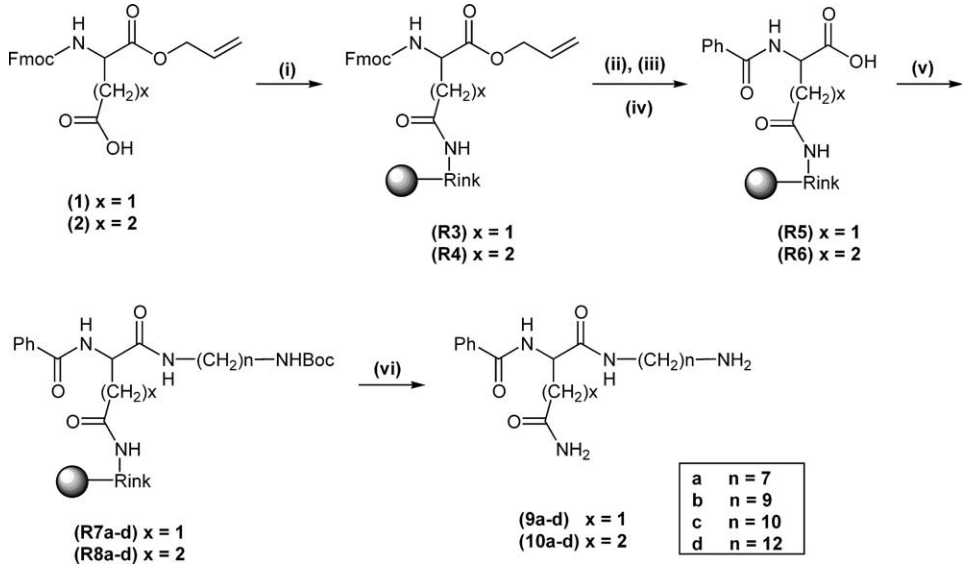
For preparation of the polyamine derivatives, **17** and **18**, the N^1, N^4, N^9-Boc_3-spermine **14** was required, this was prepared using the acrylonitrile approach.[14] Michael addition of 1,4-diaminobutane with acrylonitrile afforded the aminonitrile derivative **11** in 83% yield, which was subsequently reacted with di-*tert*-butylcarbonate to give the Boc protected dinitrile **12**[15] in 91%. Raney-nickel/H_2 reduction gave the extended N^4, N^9-Boc_2-spermine **13**, which was converted into the required N^1, N^4, N^9-Boc_3-spermine **14** on reaction with BOC-ON (Scheme 2).

The polyamine derivatives, **17** and **18**, were prepared in a similar manner as that described for the diamine derivatives **9** and **10**, by coupling of the Boc-protected spermine **14** at the C-terminus of the aspartic and glutamic acid derivatives **R5** and **R6**, after preactivation with TBTU/HOBt (Scheme 3). The spermine derivatives of asparagine and glutamine, **17** and **18**, were obtained in quantitative yields, after purification by aqueous HCl/organic extractions (Table 2).

To increase further the number of cationic sites for potential interaction with TAR, the basic amino acid, lysine, was introduced at the N-terminus of the resin-bound amino acid derivatives **R3** and **R4** (Scheme 4). Fmoc-Lys(Boc)-OH was coupled to the N-terminus to give **R19** and **R20** then, following the previously described methodology, the diamine and polyamine derivatives **23–25** were obtained in good yields (Table 2).

Biological Assay

The amines **9**, **10**, **17**, **18**, **23–25** were evaluated for tat-TAR binding inhibitory activity using the Fusion Induced Gene Stimulation (FIGS) assay,[16] a high throughput cellular assay used to study inhibition of tat–TAR or viral fusion. Briefly the system consists of the HIV-donor lymphocyte line Hut/4–3 which constitutively produces infectious virus and expresses HIV-Env on its surface. A second, adherent cell line SX22–1 is HeLa derived but expressing CD4 and CXCR4 on its surface. As a stable endogenous reporter SX22–1 cells



Scheme 1. Reagents and conditions: (i) (a) TBTU, DIPEA, DMF, 3 min (b) Rink amide resin; (ii) 20% piperidine, DMF, 1 h; (iii) $PhCO_2H$, TBTU/HOBt, DIPEA, o/n; (iv) $(Ph_3P)Pd$, CH_2Cl_2/AcOH/*N*-methyl morpholine (2:1:37 v/v/v), 12 h; (v) TBTU/HOBt, DIPEA, DMF, H_2N–$(CH_2)n$-NHBoc, o/n; (vi) 10% TFA, 1% TIS, CH_2Cl_2, 30 min.

Table 1. HRMS data and yields for compounds **9** and **10**

Compound	x	R	Yield (%)	HRMS $(M+H)^+$
9a	1	$NH(CH_2)_7NH_2$	80	349.2240
9b	1	$NH(CH_2)_9NH_2$	77	377.2553
9c	1	$NH(CH_2)_{10}NH_2$	97	391.2704
9d	1	$NH(CH_2)_{12}NH_2$	75	419.3027
10a	2	$NH(CH_2)_7NH_2$	88	363.2393
10b	2	$NH(CH_2)_9NH_2$	82	391.2707
10c	2	$NH(CH_2)_{10}NH_2$	93	405.2862
10d	2	$NH(CH_2)_{12}NH_2$	98	433.3178

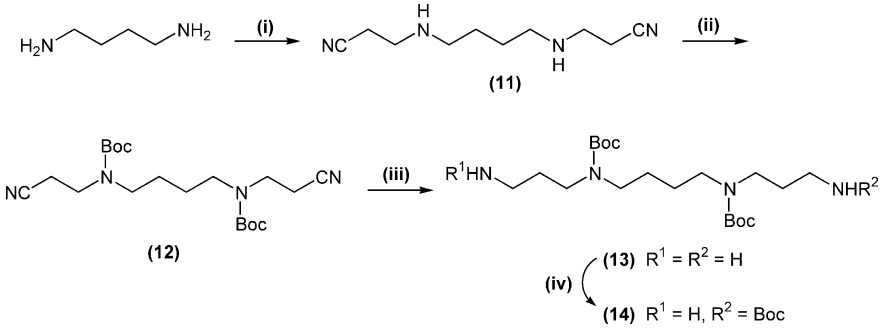
Table 2. HRMS data and yields for polyamine derivatives

Compound	Yield (%)	HRMS $(M+H)^+$
17	100	421.2925
18	100	435.3081
23	85	667.4286
24	100	681.4456
25	84	623.3929

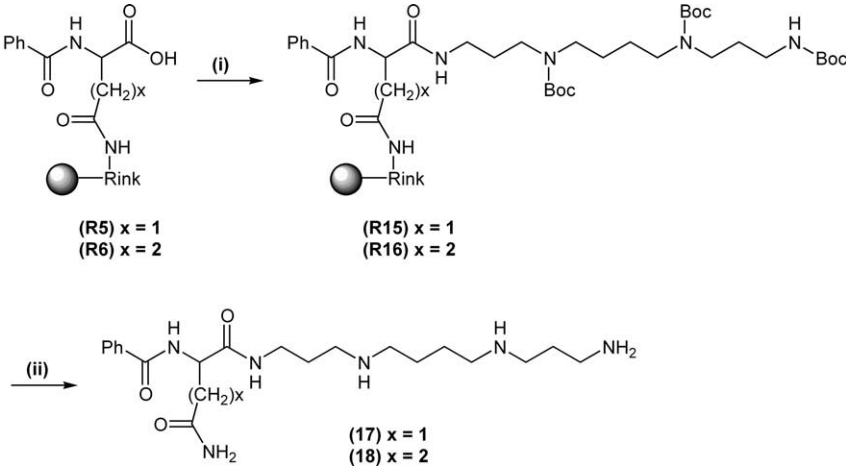
contain a lacZ gene which is controlled by the HIV-1 promoter. With this construct SX22–1 cells express β-galactosidase only after they get infected with HIV. Upon simple cocultivation of Hut/4–3 cells and SX22–1 cells membrane fusion occurs rapidly by the gp120/CD4 interaction, and the subsequent HIV gene induction can be quantitatively assessed via β-Gal activity using a colorimetric readout (ONPG to ONP conversion at 425 nm). When co-cultivation is done in the presence of an Env inhibitor, it will prevent cell fusion and no Tat transactivator will reach the reporter cells. Consequently cells remain white with no multinucleated syncytia forming. In contrast, in the presence of Tat-inhibitors those Env-driven syncytia do develop but no subsequent viral gene induction and no ONPG conversion will be detected. We used the system to quantitatively assess the potency of inhibitor candidates via their impact on cell fusion and subsequent HIV gene induction as a function of inhibitor concentration. Fusion was scored as 'visible syncytia' with at least 4–5 nuclei; a significant inhibitory activity was scored when the ONP readout was reduced by at least 30%.

Results

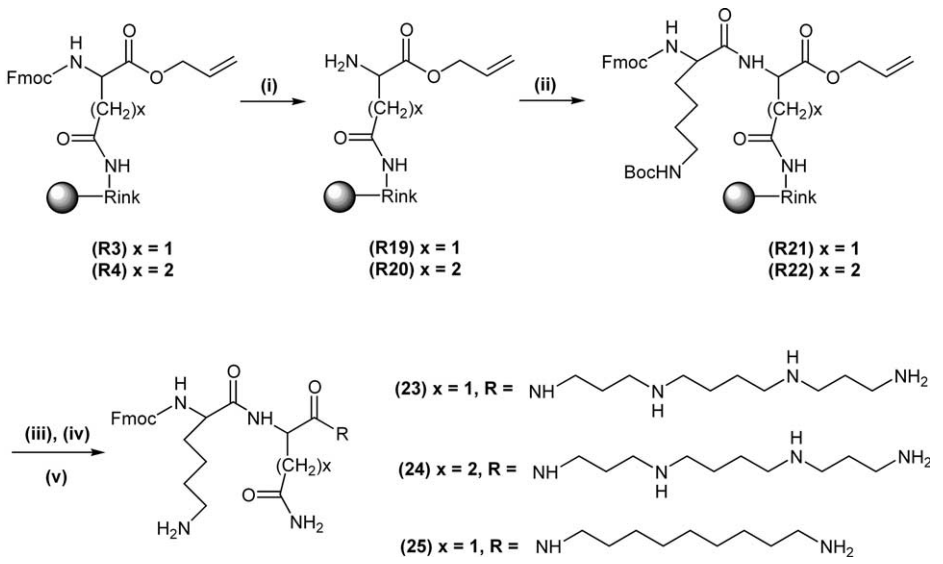
The amines **9**, **10**, **17**, **18**, **23**–**25** were evaluated at 100 μM final concentration with DMSO used as the control. As can be seen from Figure 1, the polyamines **23**–**25**, which contain the Fmoc-Lys at the N-terminus were the most active with a moderate 40% reduction of tat–TAR inhibition compared with the control. Spectroscopy for **23**–**25** was performed to confirm the absorption observed 425 nm was not owing to the Fmoc moiety. No absorption was observed for **23**–**25** at this wavelength confirming the absorption observed in the assay was a result of tat–TAR binding inhibition rather than the Fmoc moiety.



Scheme 2. Reagents and conditions: (i) $H_2C{=}CHCN$, EtOH, 20 h; (ii) $(Boc)_2O$, MeOH, 24 h; (iii) Raney Ni, EtOH/THF (4:1 v/v), H_2, 7 h; (iv) Boc-ON, THF, 4 h.



Scheme 3. Reagents and conditions: (i) TBTU/HOBt, DIPEA, DMF, **14**, o/n; (ii) 10% TFA, 1% TIS, CH_2Cl_2, 30 min.



Scheme 4. Reagents and conditions: (i) 20% piperidine, DMA, 30 min; (ii) Fmoc-Lys(Boc)-OH, TPTU/HOBt, DIPEA, DMF; (iii) $(Ph_3P)Pd$, CH_2Cl_2/AcOH/*N*-methyl morpholine (2:1:37 v/v/v), 12 h; (v) TBTU/HOBt, DIPEA, DMF, H_2N–$(CH_2)n$-NHBoc or **14**, o/n; (vi) 10% TFA, 1% TIS, CH_2Cl_2, 30 min.

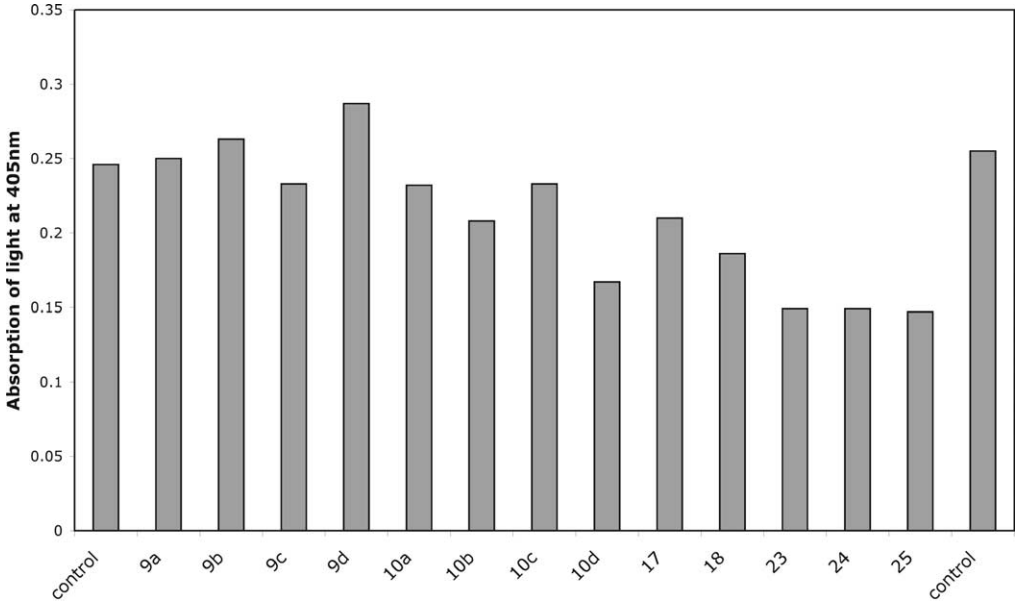


Figure 1. Percent inhibition of tat–TAR binding at inhibitor concentration of 100 μM. Absorption at 405 nm in a standard ONP Assay serves as readout to determine compound activity in comparison to the untreated control (left and right: 0.25 arbitrary Absorption units). Reduction of the reading at 405 nm correlated with suppression of HIV gene activity and was scored significant only when reaching a reduction of at least 20%.

To ascertain whether the observed inhibition of HIV-mediated fusion caused by compounds **23**–**25** were the result of their effect on membrane function rather than cytotoxicity, the compounds were further evaluated using reporter cells transfected with viral DNA (transfection-FIGS assay). With this complementary assay viral expression occurs within the cells with fusion occurring as a secondary event. Compounds **23**–**25** were assayed at seven concentrations (ranging from 0.5 to 500 μM), eight points per concentration were assessed in a 48-h transfection-FIGS assay. Whereas the ONPG reading gave IC_{50} values of 205, 159 and 158 μM, respectively, microscopic inspection of X-gal stained cells revealed that no cells were left $\geq IC_{50}$ concentrations, indicating that the observed effect correlated with cell loss due to presence of the respective compounds. Likely reasons include cytotoxicity or charge-related surface effects, which lead to detachment of cells from the surface and loss for the ONP-detection step. It has been reported that when adding polycations, adherent cells can spontaneously detach from the plastic well (yet stay alive!), as their anchoring heparansulfate proteoglycans (HSPG) become recruited. A similar effect can be observed with cationic detergents, cationic polymers or following addition of EDTA which removes calcium bridges formed by HSPG. No effect on fusion was observed at sub-toxic concentrations, with **25** appearing to enhance syncytia formation.

In conclusion, the polyamines **23**–**25** do show an overall effect on viral function, particularly in their ability to

fuse cells. However, the results from the transfection-FIGS assay would suggest that cytostatic events, resulting from the compounds ability to interfere with normal cellular processes, are also involved, or, alternatively, processes that lead to cell detachment and hence loss prior to colorimetric activity assessment.

Experimental

^1H and ^{13}C NMR spectra were recorded with a Brucker Avance DPX300 spectrometer operating at 300 and 75 MHz, respectively, with Me_4Si as internal standard, using deuterated methanol purchased from Goss unless stated otherwise. Low-resolution electrospray (ES) and atmospheric pressure chemical ionisation (APCI) mass spectra were recorded on a Fision VG Platform II spectrometer. High-resolution mass spectra were determined by the EPSRC Mass Spectrometry Centre, Swansea, UK, using electrospray. Tlc were carried out on precoated silica plates (kiesel gel 60 F_{254}) from BDH with visualisation via UV light at 254 nm or ninhydrine solution purchased from sigma. Rink amide resin was purchased from Novabiochem and empty columns for combinatorial chemistry were purchased from Pharmacia Biotech. Dry solvents were purchased from Aldrich or Fluka and all were used as supplied. All the reactions were carried out under nitrogen using anhydrous solvents. Cell media, media supplements, PBS-buffer, HEPES and serum were purchased from Life Technologies (Paisley, UK).

General methods

Coupling to the resin. A DMF solution containing a large excess of substrate (5 mmol), DIPEA (8 mmol) and TBTU (5 mmol) was stirred for 3 min for preactivation. This mixture was added to the Rink Amide MBHA resin (potential loading 1 mmol) and the suspension shaken for 1 h. The resin was then washed using the following series of solvents: DMF, H_2O, CH_2Cl_2, ^{i}PrOH and finally MeOH.

***N*-Acylation [benzoyl or Fmoc-Lys(Boc)-OH].** The loaded resin (102 mg, 0.06 mmol) was suspended in DMF (3 mL) in the reactor and a solution of either benzoic acid or Fmoc-Lys(Boc)-OH (0.18 mmol), TPTU (53 mg, 0.18 mmol), HOBt (24 mg, 0.18 mmol) and DIPEA (26 mg, 0.2 mmol) in DMF (3 mL) were added, then the mixture was shaken overnight. Extensive washings with DMF (2×3 min), H_2O (2×3 min), CH_2Cl_2 (2×3 min) and MeOH (2×3 min) left the resin clean of any excess reagent.

Allyl deprotection. The dried loaded resin (0.12 mmol) was resuspended in a degassed solution of acetic acid and *N*-methylmorpholine in CH_2Cl_2 (2:1:37 v/v/v, 3 mL) then tetrakis(triphenylphosphine)palladium (0.096 mmol) added under a nitrogen atmosphere. After treatment for 12 h, the resin was washed with CH_2Cl_2 (3×1 min), DMF (3×1 min), a solution of sodium diethyldithiocarbamate (0.05 M) containing 0.5% DIPEA in DMF (2×1 min), DMF (2×1 min) and finally CH_2Cl_2 (3×1 min).

Amine (diamine/polyamine) coupling. A sample of loaded resin (0.3 mg, 0.15 mmol) was subjected to preactivation with TBTU/HOBt (1:1 wt/wt, 192/81 mg) in the presence of DIPEA (0.20 mL, 1.2 mmol) with DMF (3 mL) as the solvent, for 3 min. Then a 5-fold excess of the amine (0.75 mmol) was added and the resin shaken overnight. Monoprotected diamines used in the preparation of compounds **9** and **10** were a gift of Dr. Richard Weaver, Cardiff University and were made by treating the corresponding diamines with BOC-ON under high dilution conditions.

Fmoc deprotection. The resin (150 mg, 0.086 mmol) was shaken with a solution of piperidine/DMA (1:4 v/v, 6 mL) six times for a period of 2 min each. The resin was then washed with ^{i}PrOH (3×1 min), DMA (2×1 min), ^{i}PrOH (3×1 min), DMA (2×1 min), H_2O and finally MeOH.

General procedure for cleavage from the resin (9, 10, 17, 18, 23, 24, 25). The loaded resin containing the asparagine or glutamine derivative was placed in the reactor and a solution of 10% TFA, 1% TIS in CH_2Cl_2 (6 mL) was added. The suspension was shaken for 30 min and the solvent was drained and kept. Additional washings with 5% TFA, 1% triethylsilane in CH_2Cl_2 (2×10 mL) were carried out. The washings from the resin were combined and evaporated and the resulting residue azeotroped with MeOH to remove any traces of TFA. The product was purified by initial washing with diethyl ether followed by CH_2Cl_2/H_2O and $EtOAc/H_2O$ extractions, or aqueous HCl/organic extractions, the aqueous layers were combined, concentrated and the product dried in vacuo. In some cases the products were further purified by flash column chromatography (see individual experimental for purification method).

The numbering system used for the NMR analysis is shown in Figure 2.

***N*^{1} - Heptylamino - 2 - (phenylcarboxamido)succinamide (9a).** $C_{18}H_{28}N_4O_3$, purification by flash column chromatography (CH_2Cl_2, MeOH, 1:1 v/v), yield: 80%. ^1H NMR δ 7.85 (d, J = 7.0 Hz, 2, H-7, 11), 7.50 (m, 3, H-8, 9, 10), 4.91 (t, J = 7.5 Hz, 1, H-2), 3.22 (t, J = 6.8 Hz, 2, H-12), 2.90 (t, J = 7.6 Hz, 2, H-18), 2.82 (dd, J = 6.1, 7.3 Hz, 2, H-3), 1.64 (m, 2), 1.53 (m, 2), 1.37 (m, 6); ^{13}C NMR δ 175.6 (C, C-4), 173.6 (C, C-1), 170.4 (C, C-5), 135.4 (C, C-6), 133.4 (CH, C-9), 130.0 (CH, C-8, 10), 128.9 (CH, C-7, 11), 52.9 (CH, C-2), 41.0 (CH_2, C-18), 40.8 (CH_2, C-12), 38.3 (CH_2, C-3), 30.5, 30.0, 28.8, 27.8, 27.6 (5×CH_2); anal. HRMS $C_{18}H_{29}N_4O_3$ $(M+H)^+$ requires 349.2239, found 349.2240.

***N*^{1}-Nonylamino-2-(phenylcarboxamido)succinamide (9b).** $C_{20}H_{32}N_4O_3$, purification by flash column chromatography (CH_2Cl_2/MeOH, 1:1 v/v), yield: 77%. ^1H NMR δ 7.83 (d, J = 7.3 Hz, 2, H-7, H-11), 7.51 (m, 1, H-9), 7.42 (m, 2, H-8, H-10), 4.95 (t, J = 7.5 Hz, 1, H-2), 3.16 (t, J = 6.1 Hz, 2, H-12), 2.86 (m, 4, H-3, H-20), 1.60 (m, 2, H-13), 1.46 (m, 2), 1.27 (m, 10); ^{13}C NMR δ 175.6 (C, C-4), 173.6 (C, C-1), 170.3 (C, C-5), 135.5 (C, C-6), 133.4 (CH, C-9), 130.0 (CH, C-8, C-10), 128.9 (CH,

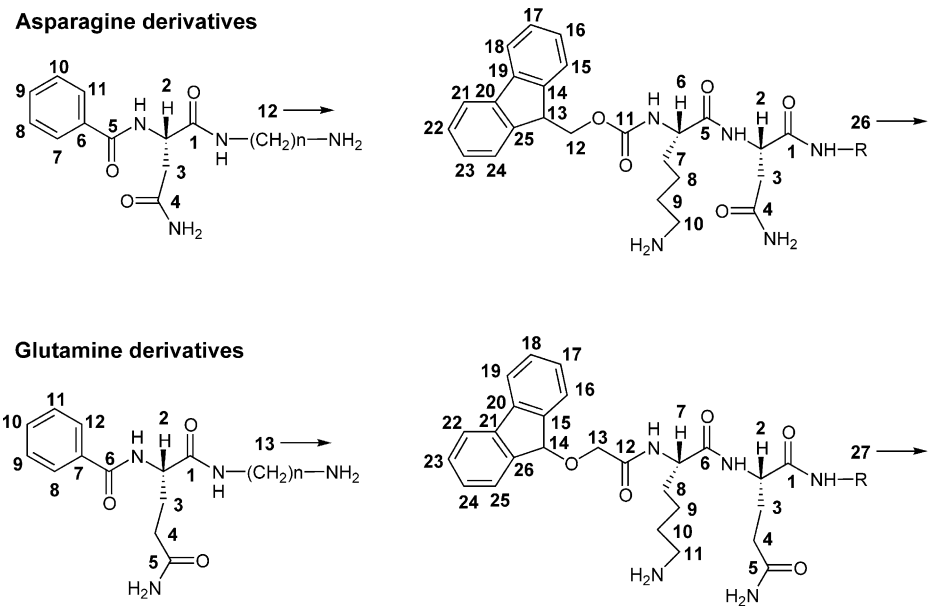


Figure 2. Numbering systems used for assignment of the NMR analysis of asparagine and glutamine derivatives.

C-7, C-11), 52.9 (CH, C-2), 41.1 (CH_2, C-20), 40.9 (CH_2, C-12), 38.4 (CH_2, C-3), 30.6, 30.5, 30.4, 28.9, 28.1, 27.7 (7×CH_2); anal. HRMS $C_{20}H_{33}N_4O_3$ $(M+H)^+$ requires 377.2552, found 377.2553.

***N*¹-Decylamino-2-(phenylcarboxamido)succinamide (9c).** $C_{21}H_{34}N_4O_3$·HCl, purification by aqueous HCl/organic extractions, yield: 97%. ^{1}H NMR δ 7.85 (d, J = 7.25 Hz, 2, H-7, H-11), 7.55 (m, 1, H-9), 7.50 (m, 2, H-8, H-10), 4.9o (bt, 1, H-2), 3.21 (t, J = 6.9 Hz, 2, H-12), 2.90 (t, J = 7.1 Hz, 2, H-21), 2.82 (m, 2, H-3), 1.60 (m, 2, H-13), 1.50 (m, 2), 1.35 (m, 12); ^{13}C NMR δ 175.6 (C, C-4), 173.6 (C, C-1), 170.4 (C, C-5), 135.5 (C, C-6), 133.4 (CH, C-9), 129.9 (CH, C-8, C-10), 128.9 (CH, C-7, C-11), 52.9 (CH, C-2), 41.2 (CH_2, C-21), 41.0 (CH_2, C-12), 38.4 (CH_2, C-3), 30.9, 30.8, 30.7, 30.5, 28.9, 28.2, 27.8 (8×CH_2); anal. HRMS $C_{21}H_{35}N_4O_3$ $(M+H)^+$ requires 391.2709, found 391.2704.

***N*¹ - Dodecylamino - 2 - (phenylcarboxamido)succinamide (9d).** $C_{23}H_{38}N_4O_3$·TFA, purification by H_2O/organic extractions, yield: 75%. ^{1}H NMR δ 7.81 (d, J = 7.3 Hz, 2, H-7, H-11), 7.52 (t, J = 7.35 Hz, 1, H-9), 7.44 (m, 2, H-8, H-10), 4.85 (m, 1, H-2), 3.21 (t, J = 6.9 Hz, 2, H-12), 2.90 (m, 2, H-3), 2.81 (m, 2, H-23), 1.64 (m, 2, H-13), 1.50 (m, 2), 1.30 (m, 16); ^{13}C NMR δ 175.6 (C, C-4), 173.5 (C, C-1), 170.3 (C, C-5), 135.5 (C, C-6), 133.4 (CH, C-9), 130.0 (CH, C-8, C-10), 128.9 (CH, C-7, C-11), 52.8 (CH, C-2), 41.3 (CH_2, C-23), 41.0 (CH_2, C-12), 38.3 (CH_2, C-3), 31.1, 30.9, 30.8, 30.7, 30.6, 29.4, 28.3, 27.8 (10×CH_2); anal. HRMS $C_{23}H_{39}N_4O_3$ $(M+H)^+$ requires 419.3022, found 419.3027.

***N*-1-{1-[(7-Aminoheptyl)carbamoyl]-3-carbamoylpropyl}-benzamide (10a).** $C_{19}H_{30}N_4O_3$·HCl, purification by aqueous HCl/organic extractions, yield: 88%. ^{1}H NMR δ 7.85 (d, J = 7.25 Hz, 2, H-8, H-12), 7.45 (m, 3, H-9, H-10, H-11), 4.49 (dd, J = 3.8, 4.9 Hz, 1, H-2), 3.24 (t, J = 6.7 Hz, 2, H-13), 2.90 (t, J = 7.5 Hz, 2, H-19), 2.40 (t, J = 6.8 Hz, 2, H-4), 2.15 (m, 2, H-3), 1.37–1.64 (m, 10); ^{13}C NMR δ 175.5 (C, C-5), 174.5 (C, C-1), 170.6 (C, C-6), 135.5 (C, C-7), 133.4 (CH, C-10), 129.9 (CH, C-9, C-11), 128.9 (CH, C-8, C-12), 55.9 (CH, C-2), 41.1 (CH_2, C-19), 40.6 (CH_2, C-13), 30.5, 30.0, 29.1, 28.7, 27.8, 27.6 (7×CH_2); anal. HRMS $C_{19}H_{31}N_4O_3$ $(M+H)^+$ requires 363.2396, found 363.2393.

***N*-1-{1-[(7-Aminononyl)carbamoyl]-3-carbamoylpropyl}-benzamide (10b).** $C_{21}H_{34}N_4O_3$·HCl, purification by aqueous HCl/organic extractions, yield: 82%. ^{1}H NMR δ 7.90 (d, J = 7.3 Hz, 2, H-8, H-12), 7.55 (t, J = 7.25 Hz, 1, H-10), 7.45 (m, 2, H-9, H-11), 4.50 (m, 1, H-2), 3.20 (t, J = 6.9 Hz, 2, H-13), 2.90 (t, J = 6.8 Hz, 2, H-21), 2.40 (t, J = 7.1 Hz, 2, H-4), 2.15 (m, 2, H-3), 1.58 (m, 4), 1.35 (m, 10); ^{13}C NMR δ 174.4 (C, C-5), 170.6 (C, C-1 and C-6), 135.4 (C, C-7), 133.3 (CH, C-10), 129.9 (CH, C-9, C-11), 128.9 (CH, C-8, C-12), 55.8 (CH, C-2), 41.1 (CH_2, C-21), 40.8 (CH_2, C-13), 30.7, 30.5, 30.0, 29.1, 28.9, 28.2, 27.7 (9×CH_2); anal. HRMS $C_{21}H_{35}N_4O_3$ $(M+H)^+$ requires 391.2709, found 391.2707.

***N*-1-{1-[(7-Aminodecyl)carbamoyl]-3-carbamoylpropyl}-benzamide (10c).** $C_{22}H_{36}N_4O_3$·HCl, purification by aqueous HCl/organic extractions, yield: 93%. ^{1}H NMR δ 7.90 (bs, 2, H-8, H-12), 7.50 (bs, 3, H-9, H-10, H-11), 4.55 (bs, 1, H-2), 3.15 (bs, 2, H-13), 2.90 (bs, 2, H-22), 2.50 (bs, 2, H-4), 2.20 (bs, 2, H-3), 1.25–1.65 (m, 16); ^{13}C NMR δ 175.3 (C, C-5), 174.3 (C, C-1), 170.2 (C, C-6), 135.4 (C, C-7), 133.5 (CH, C-10), 130.1 (CH, C-9, C-11), 129.0 (CH, C-8, C-12), 55.8 (CH, C-2), 41.3 (CH_2, C-22), 40.9 (CH_2, C-13), 31.0 (CH_2, C-21), 30.7, 30.4, 29.2, 29.1, 28.3, 27.9 (9×CH_2); anal. HRMS $C_{22}H_{37}N_4O_3$ $(M+H)^+$ requires 405.2865, found 405.2862.

***N*-1-{1-[(7-Aminododecyl)carbamoyl]-3-carbamoylpropyl}benzamide (10d).** $C_{24}H_{40}N_4O_3$·HCl, purification by aqueous HCl/organic extractions, yield: 98%. ^{1}H NMR δ 7.90 (d, J = 7.2 Hz, 2, H-8, H-12), 7.55 (t, J = 7.1 Hz,

1, H-10), 7.50 (m, 2, H-9, H-11), 4.50 (m, 1, H-2), 3.20 (t, $J=6.9$ Hz, 2, H-13), 2.85 (t, $J=6.8$ Hz, 2, H-24), 2.37 (t, $J=7.1$ Hz, 2, H-4), 2.15 (m, 2, H-3), 1.65 (m, 2), 1.53 (m, 2), 1.27 (m, 16); ^{13}C NMR δ 174.3 (C, C-5), 170.5 (C, C-1 and C-6), 135.4 (C, C-7), 133.3 (CH, C-10), 129.9 (CH, C-9, C-11), 128.9 (CH, C-8, C-12), 55.8 (CH, C-2), 41.2 (CH_2, C-24), 40.8 (CH_2, C-13), 31.0–30.6 ($8\times CH_2$), 29.1, 28.9, 28.3, 27.8 ($4\times CH_2$); anal. HRMS $C_{24}H_{41}N_4O_3$ $(M+H)^+$ requires 433.3188, found 433.3178.

N^4,N^4-Di(*tert*-butyloxycarbonyl)-N^1, N^4-di-(3-aminopropyl)-1,4-butanediamine (13). To a solution of **12**[17] (3.16 g, 16.0 mmol) in a mixture of EtOH and THF (128:32 mL) was added Raney-nickel (3.7 g, 32.0 mmol, 50% suspension in H_2O) and aqueous 2 N NaOH (19 mL), then the reaction stirred under a H_2 atmosphere at room temperature for 7 h. The catalyst was removed by filtration and the filtrate concentrated under reduced pressure. The resulting residue was dissolved in CH_2Cl_2 (100 mL) and washed with H_2O (30 mL), then the aqueous layer back-extracted with CH_2Cl_2 (3×30 mL). The organic fractions were combined, dried ($MgSO_4$) and concentrated under reduced pressure to give the product as a yellow viscous oil (2.82 g, 87%). ^{1}H NMR ($CDCl_3$) δ 3.21 (m, 4, H-3, H-10), 3.14 (m, 4, H-5, H-8), 2.66 (t, $J=6.7$ Hz, 4, H-1, H-12), 1.68 (m, 4, $2\times NH_2$, ex), 1.61 (t, $J=6.8$ Hz, 4, H-2, H-11), 1.50–1.68 (m, 4, H-6, H-7), 1.42 (s, 18, $2\times C(C\underline{H}_3)_3$); ^{13}C NMR ($CDCl_3$) δ 155.8 (C=O), 79.4 (C, $\underline{C}(CH_3)_3$), 46.6 (CH_2, C-3, C-10), 44.0 (CH_2, C-5, C-8), 39.2 (CH_2, C-1, C-12), 31.8 (CH_2, C-2, C-11), 28.5 (CH_3, $2\times C(\underline{C}H_3)_3$), 25.6 ($CH_2$, C-7, C-8). LRMS ($ES^+$) m/z 403 ($M+H^+$, 30%), 303 ($M+2H^+-{}^tBu^+$, 12%), 202 ($M+2H^+$)/2, 65%), 146 (($M+4H^+-2{}^tBu^+$)/2, 100%), 124 (55%), 102 (Boc + H^+, 30%).

N^4, N^4, N^{12}-Tri(*tert*-butyloxycarbonyl)-N^1, N^4-di-(3-aminopropyl)-1,4-butanediamine (14). A solution of BOC-ON (1.31 g, 5.3 mmol) in THF (30 mL) was added dropwise over 4 h to a solution of **13** (2.14 g, 5.3 mmol) in THF (100 mL). The reaction mixture was then concentrated under reduced pressure and the resulting residue purified by flash column chromatography (CH_2Cl_2/MeOH/Et_3N, 96:4:0 v/v increasing to 94:4:2) to give the product as a brown oil (0.92 g, 36%). ^{1}H NMR δ 3.21 (Bs, 8, H-3, H-5, H-10, H-12) 3.02 (bt, 2, H-1), 2.64 (bt, 2, H-8), 1.7 (m, 4, H-2, H-11), 1.51 (m, 4, H-6, H-7), 1.48 (s, 18, $2\times C(C\underline{H}_3)_3$), 1.43 (s, 9, $C(C\underline{H}_3)_3$); LRMS (ES^+) m/z 503 ($M+H^+$, 100%), 403 ($M+H^+-$Boc, 40%), 303 ($M+H^+-2$Boc, 30%).

N^1-Spermine-2-(phenylcarboxamido)succinamide (17). $C_{21}H_{36}N_6O_3\cdot3HCl$, purification by aqueous HCl/organic extractions, yield: 100%. ^{1}H NMR δ 7.95 (d, $J=7.3$ Hz, 2, H-7, H-11), 7.61 (bt, 1, H-9), 7.55 (m, 2, H-8, H-10), 4.95 (bt, 1, H-2), 3.41 (bs, 2, H-12), 3.11 (bm, 12), 2.20 (bs, 2, H-13), 1.98 (bs, 2), 1.88 (bs, 4); ^{13}C NMR δ 176.5 (C, C-1), 174.7 (C, C-4), 170.5 (C, C-5), 135.1 (C, C-6), 133.7 (CH, C-9), 130.2 (CH, C-8, C-10), 129.2 (CH, C-7, C-11), 52.9 (CH, C-2), 48.8, 48.7 (CH_2, C-14, C-21), 46.7, 46.4 (CH_2, C-16, C-19), 38.6, 38.5, 37.6 (CH_2, C-3, C-12, C-23), 27.8, 25.8 (CH_2, C-13, C-22), 24.7 (CH_2, C-17, C-18). anal. HRMS $C_{21}H_{37}N_6O_3$ $(M+H)^+$ requires 421.2927, found 421.2925.

N^1-(1-{[3-({4-[(3-Aminopropyl)amino]butyl}amino)propyl]carbamoyl}-3-carbamoylpropyl)benzamide (18). $C_{22}H_{38}N_6O_3\cdot3HCl$, purification by aqueous HCl/organic extractions, yield: 100%. ^{1}H NMR δ 7.90 (d, $J=7.1$ Hz, 2, H-8, H-12), 7.45 (m, 3, H-9, H-10, H-11), 4.41 (bt, 1, H-2), 3.25 (m, 2, H-13), 2.90 (m, 10), 2.45 (bt, 2, H-4), 1.70–1.90 (m, 10); ^{13}C NMR δ 179.6 (C, C-5), 175.4 (C, C-1), 170.7 (C, C-6), 135.2 (C, C-7), 133.6 (CH, C-10), 130.1 (CH, C-9, C-11), 129.2 (CH, C-8, C-12), 56.1 (CH, C-2), 48.7, 48.6, 46.8, 46.5, 46.4, 38.5, 38.4, 37.5, 28.7, 27.9, 25.8, 24.7 ($12\times CH_2$). anal. HRMS $C_{22}H_{39}N_6O_3$ $(M+H)^+$ requires 435.3083, found 435.3081.

N^1-[3-({4-[(3-Aminopropyl)amino]butyl}amino)propyl]-2-[(5-amino-1-{[9*H*-9-fluorenylmethoxy]carboxamido}pentyl)carboxamido]succinamide (23). $C_{35}H_{54}N_8O_5\cdot3HCl$, purification by aqueous HCl/organic extractions, yield: 85%. ^{1}H NMR δ 7.80 (d, $J=7.7$ Hz, 2, H-18, H-21), 7.7 (d, $J=8.0$ Hz, 2, H-15, H-24), 7.30 (m, 4, H-16, H-17, H-22, H-23), 4.70 (bs, 1, H-2), 4.30 (m, 2, H-12), 4.20 (bs, 1, H-13), 4.00 (bs, 1, H-6), 3.40 (bs, 1, H-26), 2.97 (m, 12), 1.95 (bs, 12), 1.45 (bs, 2); ^{13}C NMR δ 175.6 (C, C-5), 174.4 (C, C-4), 173.1 (C, C-1), 161.7 (C, C-11), 145.7 (C, C-14, C-25), 143.1 (C, C-19, C-20), 129.3 (CH, C-17, C-22), 128.7 (CH, C-16, C-23), 126.7 (CH, C-15, C-24), 121.4 (CH, C-18, C-21), 68.5 (CH2, C-12), 57.2 (CH, C-6), 53.5, 52.3 (CH, C-2, C-13), 48.8, 48.7, 48.6, 46.6, 46.4, 40.9, 38.4, 37.4, 32.2, 28.5, 27.7, 25.7, 24.7, 24.0 ($15\times CH_2$). anal. HRMS $C_{35}H_{55}N_8O_5$ $(M+H)^+$ requires 667.4295, found 667.4286.

N^1-[3-({4-[(3-Aminopropyl)amino]butyl}amino)propyl]-2-[(5-amino-1--{[9*H*-9-fluorenylmethoxy]carboxamido}pentyl)carboxamido]pentanediamine (24). $C_{36}H_{56}N_8O_5\cdot3HCl$, purification by aqueous HCl/organic extractions, yield: 100%. ^{1}H NMR δ 7.80 (d, $J=7.6$ Hz, 2, H-19, H-22), 7.70 (d, $J=8.1$ Hz, 2, H-16, H-25), 7.40 (m, 4, H-17, H-18, H-23, H-24), 4.40 (m, 3, H-7, H-27), 4.25 (m, 1, H-2), 4.15 (m, 1, H-14), 3.30 (bt, 2, H-38), 3.00 (m, 12), 1.5–2.48 (bm, 16); ^{13}C NMR δ 179.0 (C, C-6), 176.0 (C, C-5), 175.0 (C, C-1), 159.0 (C, C-12), 145.6 (C, C-15), 145.5 (C, C-26), 142.9 (C, C-20, C-21), 129.3 (CH, C-18, C-23), 128.7 (CH, C-17, C-24), 126.6 (CH, C-16, C-25), 121.4 (CH, C-19, C-22), 68.4 (CH_2, C-13), 57.0 (CH, C-7), 55.1 (CH, C-2), 48.6 (CH_2), 48.5 (CH, C-14), 46.6, 46.3, 40.9, 38.4, 37.5, 37.4, 32.8, 28.5, 27.7, 25.7, 24.6, 24.3 ($13\times CH_2$). anal. HRMS $C_{36}H_{57}N_8O_5$ $(M+H)^+$ requires 681.4452, found 681.4456.

N^1-(9-Aminononyl)-2-[(5-amino-1-{[9*H*-9-fluorenylmethoxy]carboxamido}pentyl) carboxamido]succinamide (25). $C_{34}H_{50}N_6O_5\cdot2HCl$, purification by aqueous HCl/organic extractions, yield: 84%. ^{1}H NMR δ 7.85 (d, $J=7.7$ Hz, 2, H-18, H-21), 7.70 (m, 2, H-15, H-24), 7.35 (m, 4, H-16, H-17, H-22, H-23), 4.80 (m, 1, H-2), 4.30 (m, 2, H-12), 4.20 (m, 1, H-13), 4.05 (m, 1, H-6), 2.90 (m, 8), 1.95 (bs, 12), 1.1–1.8 (bm, 20); ^{13}C NMR δ 177.5 (C, C-5), 176.1 (C, C-4), 174.1 (C, C-1), 159.1 (C, C-11), 145.6 (C, C-14, C-25), 142.9 (C, C-19, C-20), 129.3 (CH, C-17, C-22), 128.6 (CH, C-16, C-23),

126.7 (CH, C-15, C-24), 121.4 (CH, C-18, C-21), 68.7 (CH_2, C-12), 57.5 (CH, C-6), 57.4 (CH, C-13), 52.0 (CH, C-2), 41.2, 41.0, 40.9, 30.6, 30.5, 30.45, 30.41, 28.9, 28.5, 28.2, 27.8, 24.0 ($14\times CH_2$). anal. HRMS $C_{34}H_{51}N_6O_5$ $(M+H)^+$ requires 623.3921, found 623.3929.

Cellular trans-activation assay. FIGS-assays were performed as described.[16] Briefly, the HeLaT4-derived reporter cell line SX 22–1 was cocultivated with constitutively HIV-producing Hut/4–3 lymphocytes at a ratio of 3:1. The latter cells constitutively generate infectious virus particles. Cocultivation of the SX 22–1 reporter cells with the HIV-1 donor Hut/4–3 resulted in rapid HIV Env-mediated cell fusion within h. Thereby Tat protein from the HIV-1 donor (Hut/4–3) enters the reporter cells and induces the endogenous HIV LTR lacZ gene present in the SX22–1 cells. After overnight cocultivation the cultures were fixed. Cells were stained for cell associated β-galactosidase activity using X-gal as a substrate. For photometric quantification the fixed cultures were subsequently incubated with *ortho*-nitro-phenyl-galactopyranoside (ONPG) as β-galactosidase substrate, which was enzymatically converted to the chromophoric *ortho*-nitrophenol (ONP).

Cell culture. SX22–1 cells were cultured in DMEM with 4500 mg/mL glucose, 10% fetal bovine serum (FBS), supplemented with penicillin (500 IU/mL)/streptomycin (500 μg/mL). Confluent cultures were trypsinised and passaged 1:20. Hut/4–3 cells were maintained in RPMI, 10% FBS, supplemented with penicillin/streptomycin and 10 mM HEPES. Cells were kept at densities between 2×10^5 and 3×10^6.

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