Synthesis and Antimicrobial Evaluation of TAN-1057A/B Analogs

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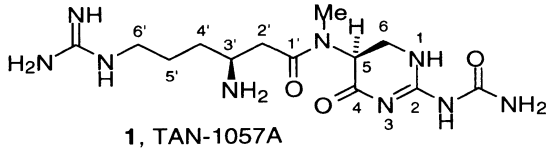
TAN-1057A~D, dipeptides isolated from bacteria *Flexibacter* sp. PK-74 and PK-176, are new antibiotics with potent antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). We describe, in detail, the synthesis of several TAN-1057A/B analogs by a convergent route featuring a new method to construct the cyclic amidinourea functional group. The biological activity of these substances against methicillin-resistant *Staphylococcus aureus* (MRSA) is reported.

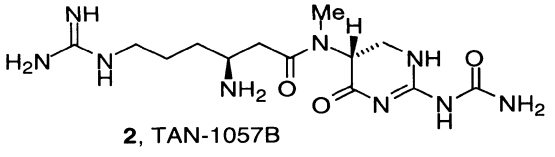
The emergence of resistance to clinically significant antibiotics has recently become a serious problem worldwide. Nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) in particular, has attracted a great deal of recent attention.[1~5] MRSA has developed resistance to most β-lactam antibiotics as well as numerous other antibiotics due to presence of the *mec A* gene.[1] MRSA produces an altered penicillin-binding protein, PBP2a, for which most clinically significant β-lactam antibiotics have low affinity. Drug discovery programs targeting new structural motifs and biochemical targets that are efficacious against MRSA have therefore become increasingly significant.

TAN-1057A~D (**1~4**), new peptide antibiotics obtained from *Flexibacter* sp. PK-74 and PK-176[6,7] by Takeda Pharmaceutical Co., Japan, were found to have potent activity against MRSA. TAN-1057A~D displayed better activity against Gram-positive bacteria than against Gram-negative bacteria. It was shown that TAN-1057A and D, which have the *S*-configuration at C5, were more active than TAN-1057 B and C which possess the *R*-configuration at this stereogenic center. There was no cross-resistance between TAN-1057 and methicillin, erythromycin and gentamicin. It is significant to note that TAN-1057A displays potent activity against all of the MRSA strains evaluated and was found to compare very favorably to vancomycin.[6] KATAYAMA, *et al.*,[6] concluded that the therapeutic effects of TAN-1057A, as determined in mice, were superior to vancomycin and imipenem, especially against MRSA. The preliminary acute toxicity (LD_{50}) data obtained for TAN-1057A was *ca.* 100 mg/kg upon intraperitoneal injection and 50 mg/kg upon intravenous injection in mice.

Preliminary mechanism of action studies revealed that, TAN-1057A did not inhibit the incorporation of tritiated



1, TAN-1057A



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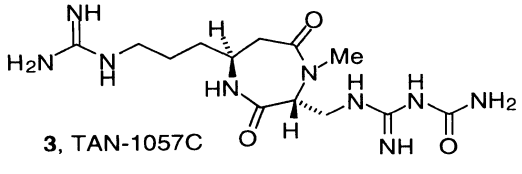
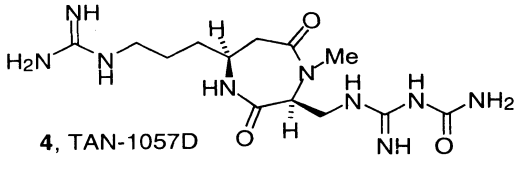
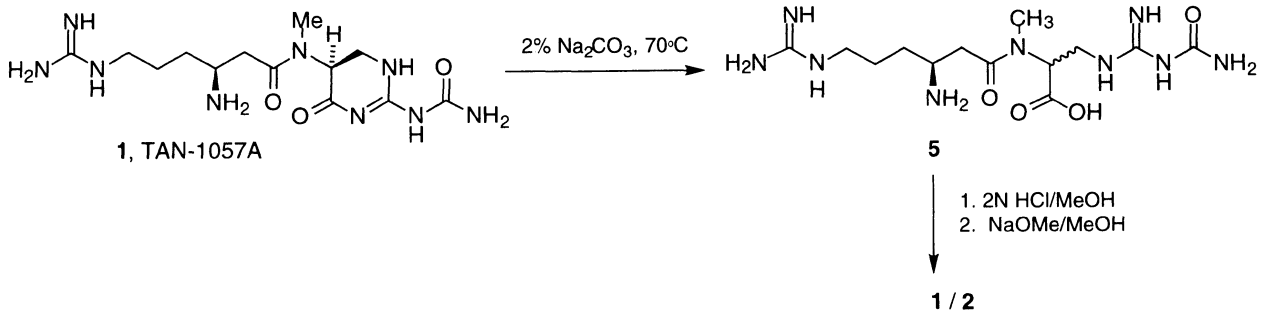


3, TAN-1057C



4, TAN-1057D

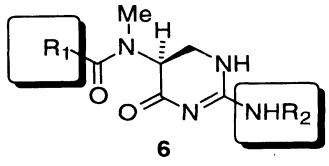
Scheme 1.



thymidine and uridine into *S. aureus* FDA 209P or *E. coli* LD-2. However, TAN-1057A inhibited the incorporation of leucine into macromolecules in these organisms at concentrations below the MIC. In addition, poly-A and poly-U-directed protein synthesis was inhibited in an *E. coli* cell-free system at 40 μg/ml and 10 μg/ml, respectively. TAN-1057A did not inhibit aminoacyl-tRNA synthetase; thus, KATAYAMA, *et al.*,[6] concluded that, the TAN-1057 series appears to interfere with protein biosynthesis after the formation of aminoacyl-tRNA. There is no published data concerning the morphological characteristics of susceptible strains treated with TAN-1057; thus, it is not presently known if TAN-1057 inhibits bacterial cell wall protein biosynthesis.

TAN-1057A and B are dipeptides consisting of β-homoarginine and a unique heterocyclic amidinourea derivative of 2,3-diaminopropionic acid. It was reported that, TAN-1057A and B gradually lost their antibacterial activities in basic aqueous solutions due to hydrolytic opening of the six-membered ring system (Scheme 1) to the biologically inactive acyclic substance **5**.[7] Hydrolysis of TAN-1057A occurs in both acidic and basic media, to afford the acyclic form (**5**) with attendant racemization of the α-amino acid stereogenic center.

Due to the unique functionality present in these structures and the possibility for the discovery of new therapeutically useful biochemical targets through mechanism of action studies on these substances, we have developed a general synthesis of TAN-1057 analogs as an initial probe of the structure/activity profile of this new antibiotic class. The synthetic approach we have developed features a new and flexible method for constructing the cyclic amidinourea.



6

Results and Discussion

Recently, we described the total synthesis of TAN-1057A~D.[8] This approach has been modified to examine the structure/functional roles of the side chains (R_1 and R_2, **6**) appended to the core cyclic amidinourea.

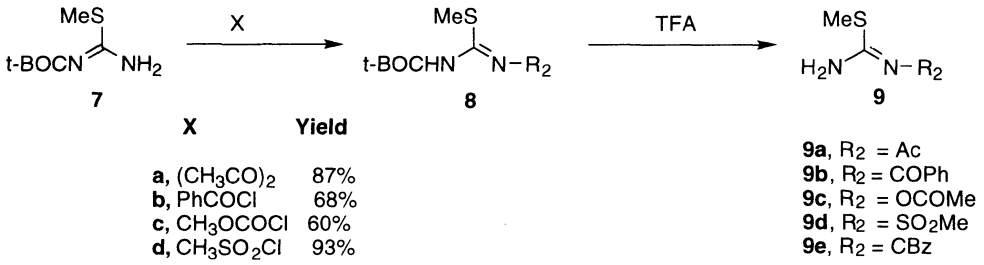
As shown in Scheme 3, dipeptide **10** was prepared as previously described[8] as a 1 : 1 mixture of stereoisomers at the C-5 stereogenic center. Condensation of **10** with the *S*-methylisothiourea derivatives **9a~e** (Scheme 2) in the presence of EDCI yielded derivatives **11a~e** (Table 1). Treatment of **11a~e** with TFA removed the BOC protecting group and the incipient amine was cyclized with triethylamine to furnish the cyclic amidinourea derivatives **12a~e**. Finally, removal of the three N–CBz groups was effected in high yield by catalytic hydrogenation yielding the TAN-1057 analogs **13a~e**.

Next, changes to the β-homoarginine side chain (R_1, **6**) were examined by the preparation of **23** and **30** as shown in Schemes 4 and 5, respectively.

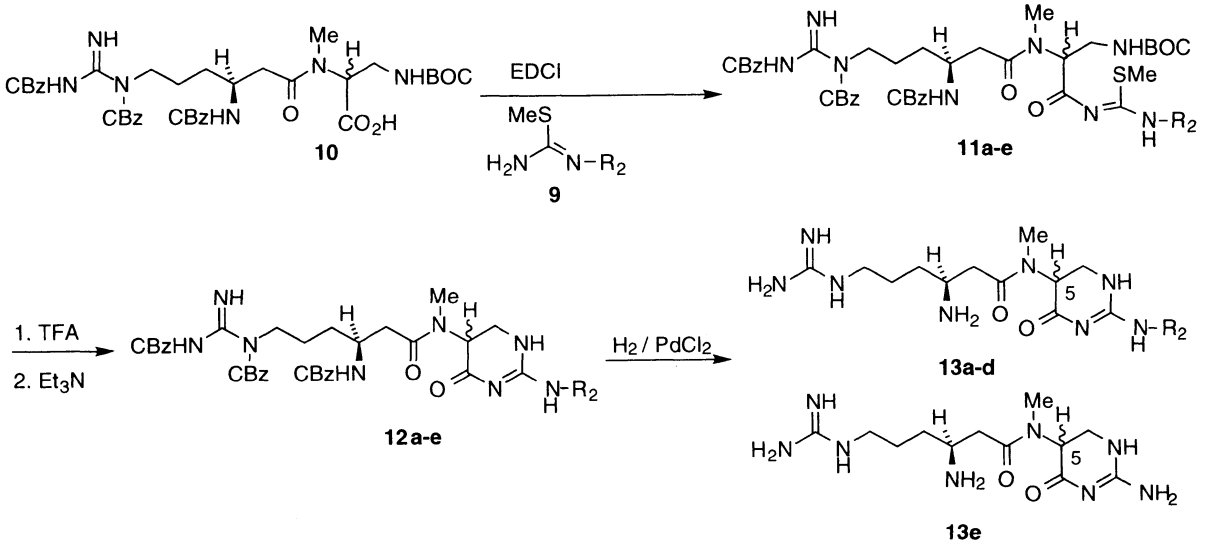
Antimicrobial Activity of TAN-1057 Synthetic Analogs

KATAYAMA, *et al.*,[6] reported that TAN-1057A was more active at pH=9 than at pH=7.[6,7] The reasons for the pH-dependence of antimicrobial activity are not clear. All synthetic compounds were first assayed against *Staphylococcus aureus* FDA 209P by the 10-fold agar disc diffusion assay on BHI plates at pH=7 and at pH=9. Compounds that displayed activity against *Staphylococcus aureus* FDA 209P were then subjected to

Scheme 2.



Scheme 3.



a more detailed antimicrobial evaluation against several strains of methicillin-sensitive *Staphylococcus aureus* (MSSA) and relevant strains of MRSA as shown in Table 2. Compounds not reported in Table 2 were inactive at the maximum concentration tested.

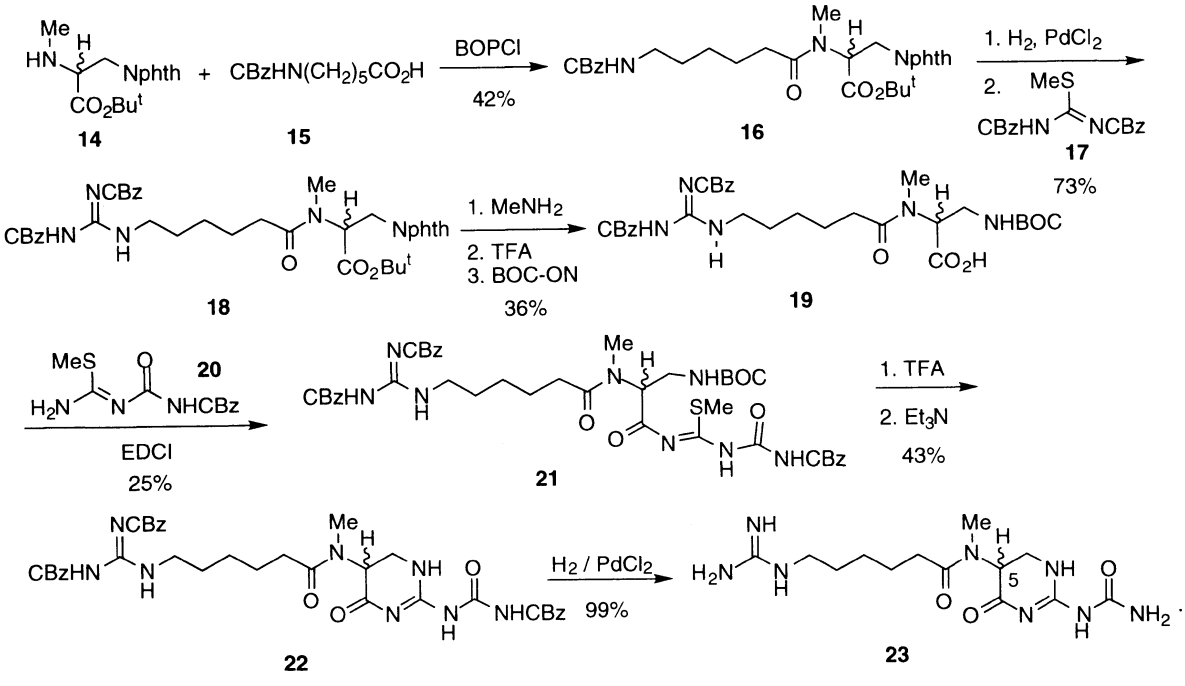
From this data, it can be seen that the TAN-1057 molecule is relatively sensitive to structural changes. Only compounds **13a** and **13c** displayed significant activity against the MRSA strains evaluated. More interesting was the complete loss of activity demonstrated by analogs **13e**, **23** and **30**. The lack of antibiotic activity displayed by these compounds indicates that 1) the acylated guanidine is essential for activity (*cf.*, **13e** with **13a** and **13c**); and 2) both basic functionality- the primary amine at C-3′ and the guanidine at C-6′ in the homoarginine side chain are essential for antibiotic activity.

The synthetic route devised herein should open the way for a more detailed, systematic study of the structure/function relationships in this new class of peptide antibiotics. In particular, the synthesis of radio-labeled versions of these substances for receptor-binding studies and structural changes to the core heterocyclic amidinourea are currently being investigated. The cyclization approach described herein to construct the unusual amidinourea moiety provides a general and flexible method for accessing a potentially important generation of anti-MRSA antibiotics. Applications of this methodology toward these goals are presently under

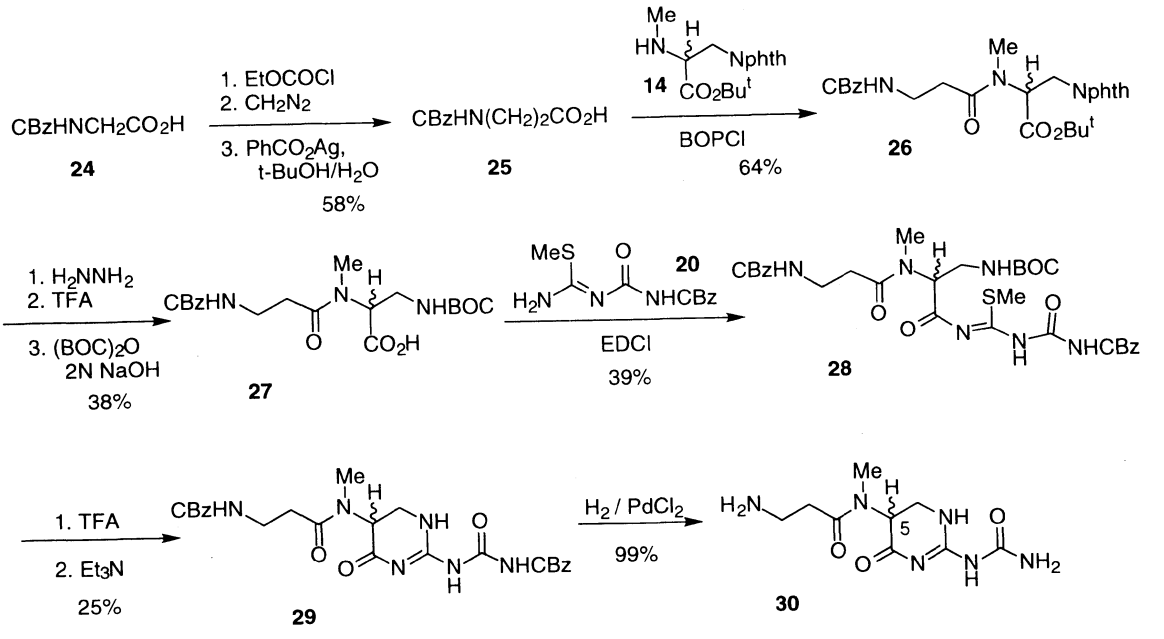
Table 1.

Entry	R_2	**11**, Yield %	**12**, Yield %	**13**, Yield %
a	Ac	52	32	99
b	COPh	39	10	99
c	COOMe	38	22	97
d	SO_2Me	34	88	99
e	CO_2CH_2Ph	52	50	99

Scheme 4.



Scheme 5.



active investigation in these laboratories and will be reported on in due course.

Materials and Methods

General

General procedures and instrumentation have been previously described.[16] Mass spectra were obtained on a 1992 Fisons VG AutoSpec. HPLC analysis of

Table 2. Minimal inhibitory concentrations (MIC's in μg/ml) of TAN-1057A and analogs.

Strain	Compound				
	TAN-1057A/B[a]	**13a**	**13c**	Imipenem*	Vancomycin
Staphylococcus aureus ATCC 29213 (MSSA)	16	16	64	≤0.25	≤0.25
Staphylococcus aureus COL 8A (MSSA)[9]	16	16	64	≤0.25	≤0.25
Staphylococcus aureus PC1[10] (MSSA)	8	8	32	≤0.25	≤0.25
Staphylococcus aureus sa ATCC 13709 (MSSA)	16	16	32	≤0.25	≤0.25
Staphylococcus aureus COL[11] (MRSA)	16	16	64	32	1
Staphylococcus aureus 76[12] (MRSA)	16	16	64	8	≤0.25
Staphylococcus aureus ATCC 33593 (MRSA)	16	8	64	8	0.5
Staphylococcus aureus sa 201 (MRSA)	16	16	64	64	0.5
Staphylococcu haemolyticus UA281[13]	32	128	256	64	1
Enterococcus faecalis ATCC 29212	32	32	64	0.5	1
Enterococcus faecium ATCC 35667	32	64	128	4	≤0.25
Enterococcus faecium BM4147[14]	32	128	64	8	>128
Enterococcus faecalis V583[13]	ND	ND	64	0.5	16
Enterococcus faecium efm 040	128	256	>256	>128	≤0.25
Escherichia coli ATCC 25922	256	128	>256	≤0.25	>128
Pseudomonas aeruginosa ATCC 27853	256	256	>256	1	>128
Solvent	H_2O/DMSO	H_2O/DMSO	MeOH	H_2O	H_2O

[a] TAN1057A/B used in this study was an equilibrium mixture of totally synthetic material prepared as described in reference 15.

TAN-1057 was carried out using a Waters 6000 pump equipped with a UV detector, utilizing an ODS, YMC Pack A-312 column, using 0.1 M phosphate buffer (pH 5.0) as the mobil phase. All the amino acids used as starting material were purchased from BACHEM Inc. Abbreviations not defined in the text: $(BOC)_2O$ = di-*tert*-butyl dicarbonate; BOC-ON = 2-(*tert*-butoxycarbonyl-oxyimino)-2-phenylacetonitile; BOP-Cl = Bis(2-oxo-3-oxazolidinyl)phosphinic chloride; DMAP = 4-dimeth ylamino pyridine; EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; NMM = 4-methyl-morpholine; TBTU = *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate. Compounds **10**, **14** and **20** were prepared as described in ref. 8. Compounds **9e** and **17** were prepared as described in reference 19.

Susceptibility Testing[17,18]

A panel of 16 bacteria was used to evaluate the antimicrobial activity of the compounds (Table 2). The MIC of each antimicrobial agent was determined using a microdilution method according to NCCLS standards.[18] Serial twofold dilutions of antibiotics were prepared in MUELLER-HINTON broth (Difco Laboratories, Detroit, Mich). Bacteria were grown to early log phase at 35°C (1 hour) in Mueller-Hinton broth and cultures were diluted to achieve a final inoculum of 5×10^5 CFU per ml. Microtiter plates were incubated at 35°C for 20 hours and then were read using a *Thermo*$_{Max}$ microplate reader (Molecular Devices, Sunnyvale, CA) and a microtiter plate reading mirror. The MIC was defined as the lowest concentration of antibiotic which inhibited the development of visible growth at the end of the incubation period. The standard reference strains were *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *E. faecium* ATCC 35667, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. *Staphylococcus aureus* sa 201 (MRSA) is a clinical isolate from Spain and *Enterococcus faecium* efm 040 is a clinical isolate from the U.S. Imipenem and vancomycin were used as antibiotic controls and values for reference strains were in accordance with the NCCLS. Although it has been reported that TAN1057A displays more potent activity in pH = 9 media versus pH = 7 media,[6,7] we have chosen to test these compounds only at pH = 7 as a more stringent indication of the capacity of the synthetic analogs to display activity under physiological pH conditions.

N-Acetyl-*N'*-butyloxycarbonyl-*S*-methylisothiourea **8a**:

To a solution of **7** (350 mg, 1.84 mmol, 1.0 eq) in CH_2Cl_2 (5.0 ml) was added acetic anhydride (190 μl/207 mg, 1.1 mmol, 1.1 eq) and TEA (388 μl, 2.76 mmol, 1.5 eq). The mixture was stirred for 16 hours at room temperature. The resulting mixture was diluted with CH_2Cl_2 (50 ml), washed with brine, dried over Na_2SO_4, filtered and concentrated. Purification *via* column chromatography (silica gel, CH_2Cl_2 : EtOAc, 9 : 1) provided 372 mg (87%) of **8a** as a semi-solid. ^{1}H NMR (300 MHz, $CDCl_3$ *vs.* TMS): δ 1.53 (9H, s, *t*-Butyl); 2.21 (3H, s, $COCH_3$); 2.39 (3H, s, S–CH_3); 12.45 (1H, br, D_2O exchanged, NH). IR (NaCl, film): 3090, 2986, 2926, 1726, 1650, 1584 cm^{-1}. *Anal* Calcd for $C_9H_{16}N_2O_3S$: C, 45.56; H, 6.94; N, 12.06. Found: C, 46.41; H, 6.99; N, 12.23.

N-Acetyl-*S*-methylisothiourea **9a**:

The **8a** (133 mg, 0.57 mmol) was treated with TFA (1.0 ml). The resulting mixture was stirred for 30 minutes at room temperature, evaporated to dryness and put on vacume line for 2 hours, triturated with anhydrous Et_2O to give 130 mg of **9a** as a semi-solid. This crude product was carried on without further purification.

^{1}H NMR (300 MHz, CD_3OD): δ 2.24 (3H, s, $COCH_3$); 2.71 (3H, s, S–CH_3); IR (NaCl, film): 3265, 2885, 1740, 1650 cm^{-1}.

S-Methylisothiourea **11a**:

To a solution of **10** (278 mg, 0.35 mmol, 1.0 eq), DMAP (115 mg, 0.95 mmol, 2.7 eq) and EDCI·HCl (81 mg, 0.42 mmol, 1.2 eq) in CH_2Cl_2 (2.0 ml) was added **9a** (130 mg, 0.53 mmol, 1.5 eq). The resulting mixture was stirred overnight at room temperature. Then, diluted with CH_2Cl_2 and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification *via* column chromatography (silica gel, CH_2Cl_2 : EtOAc, 7 : 3) provided 167 mg (52%) of **11a** as a semi-solid. ^{1}H NMR (300 MHz, $CDCl_3$ *vs.* TMS): δ 1.42 (9H, s, *t*-BuO); 1.71 (4H, m, 4'-H and 5'-H); 2.11 (3H, s, $COCH_3$); 2.27 (3H, s, S–CH_3); 2.52 (2H, m, 2'-H); 2.95 (3H, s, N–CH_3); 3.48 (1H, m, 6'-H); 3.71 (1H, m, 6'-H); 3.95 (3H, m, 3'-H and 6-H); 4.39 (1H, br, D_2O exchanged, N–H); 5.04 (2H, s, OCH_2Ph); 5.07 (1H, m, 5-H); 5.10 (2H, s, OCH_2Ph); 5.22 (2H, s, OCH_2Ph); 7.36 (15H, m, Ar-H); 5.88 (1H, d, J=5.7 Hz, HNCBz); 9.28 (1H, br, D_2O exchanged, NH); 9.43 (1H, br, D_2O exchanged, N–H); 12.06/12.19 (1H, br, D_2O exchanged). IR (NaCl, film): 3382, 2974, 2931, 1713, 1615, 1538 cm^{-1}. HR-MS: Calcd for ($C_{44}H_{56}N_8O_{11}S$ + H) = 905.3868. Found (M + H) = 905.3901.

Cyclization Product **12a**:

To a mixture of **11a** (90 mg, 0.103 mmol, 1.0 eq) in CH_2Cl_2 (0.5 ml) was added TFA (0.5 ml). The resulting mixture was stirred for 15 minutes at room temperature. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on *vacuo* for 2 hours and triturated with ethyl ether to give a white solid. This white solid was dissolved in THF (1.5 ml). To this solution was added triethylamine (30 μl, 0.206 mmol, 2.0 eq). After stirring the solution for 10 minutes, the solvent was evaporated. Separation *via* PTLC (silica gel, CH_2Cl_2 : EtOAc : MeOH, 4 : 1 : 0.5) provided 26 mg (32%) of **12a** as a colorless oil.

^{1}H NMR (300 MHz, CD_3OD): δ 1.60 (4H, m, 4'-H and 5'-H); 2.18 (3H, s, $COCH_3$); 2.55 (1.5H, m, 2'-H); 2.83 (1/2H, d, J=22 Hz, 2'-H); 2.91 (3H, s, N–CH_3); 3.28 (1H, m, 1'-H); 3.46 (2H, m, 6'-H); 3.95 (3H, m, 5-H and 6-H); 5.07 (2H, m, OCH_2Ph); 5.12 (2H, s, OCH_2Ph); 5.23 (2H, s, OCH_2Ph); 7.35 (15H, m, Ar-H). IR (NaCl, film): 3385, 3262, 2936, 1713, 1612, 1555 cm^{-1}; HR-MS: Calcd for ($C_{38}H_{44}N_8O_9$ + H) = 757.3309. Found (M + H) = 757.3299.

3*S*,5'*S*/*R*-3-Amino-6-[(aminoiminomethyl)amino]-*N*-(2-acetylamino-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-*N*-methyl-hexanamide **13a**:

To a solution of **12a** (13 mg, 0.016 mmol, 1.0 eq) in MeOH (0.5 ml)/CH_2Cl_2 (0.1 ml) was added $PdCl_2$ (13 mg). The reaction flask was charged with H_2 from a balloon and the mixture was hydrogenated at 1 atm of H_2 for 15 minutes. The mixture was then purged with nitrogen and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give a 2HCl salt of **13a** (6 mg, 99% yield) as an amorphous solid. ^{1}H NMR (300 MHz, D_2O): δ 1.77 (4H, m, 4'-H and 5'-H); 2.31 (3H, s, $COCH_3$); 2.87 (1H, m, 2'-H); 3.02 (1H, m, 2'-H); 3.14 (3H, s, N–CH_3); 3.27 (2H, t, J=6.3 Hz, 6'-H); 3.70 (1H, m, 3'-H); 3.97 (2H, m, 6-H); 5.16 (1H, m, 5-H); IR (KBr pellet): 3394, 3156, 2913, 1737, 1651, 1591 cm^{-1}. HR-MS (FAB): Calcd for ($C_{14}H_{26}N_8O_3$ + H) = 355.2206, Found (M + H) = 355.2204.

N-Benzoyl-*N'*-butyloxycarbonyl-*S*-methylisothiourea **8b**:

To a solution of **7** (190 mg, 1.0 mmol, 1.0 eq) in CH_2Cl_2 (5.0 ml) was added PhCOCl (128 μl/154 mg, 2.0 mmol, 2.0 eq) and TEA (308 μl, 2.2 mmol, 2.2 eq). The mixture was stirred for 16 hours at room temperature. The

resulting mixture was diluted with CH_2Cl_2 (50 ml), washed with brine, dried over Na_2SO_4, filtered and concentrated. Purification *via* column chromatography (silica gel, CH_2Cl_2 : EtOAc, 4 : 1) provided 200 mg (68%) of **8b** as a white solid. ^1H NMR (300 MHz, $CDCl_3$ *vs.* TMS): δ 1.45 (9H, s, *t*-BuO); 2.50 (3H, s, S–CH_3); 7.39 (3H, m, Ar-H); 8.09 (2H, m, Ar-H); 12.49 (1H, br, D_2O exchanged). IR (NaCl, film): 3067, 2980, 2929, 1746, 1612, 1538 cm^{-1}. mp: 99~101°C. HR-MS: *Anal* Calcd for ($C_{14}H_{18}N_2O_3S$ +H)=295.1133. Found (M+1)= 295.1110.

N-Benzoyl-*S*-methylisothiourea **9b**:

To a mixture of **8b** (160 mg, 0.54 mmol.) and anisole (0.1 ml) was added TFA (1.0 ml). The resulting mixture was stirred for 30 minutes at room temperature, evaporated to dryness and put on vacume line for 2 hours, triturated with anhydrous Et_2O to give 135 mg of **9b** as a semi-solid. This crude product was used directly in next step without further purification. ^1H NMR (300 MHz, CD_3OD): δ 2.74 (3H, m, S–CH_3); 7.55 (2H, t, J=7.8 Hz, Ar-H); 7.69 (1H, t, J=7.5 Hz, Ar-H); 8.02 (2H, d, J=7.5 Hz, Ar-H). IR (NaCl, film): 3226, 2936, 1695, 1680, 1540 cm^{-1}.

Coupling Product **11b**:

To a solution of **10** (158 mg, 0.20 mmol, 1.0 eq), DMAP (73 mg, 0.6 mmol, 3.0 eq) and EDCI·HCl (46 mg, 0.24 mmol, 1.2 eq) in CH_2Cl_2 (2 ml) was added **9b** (93 mg, 0.5 mmol, 1.2 eq). After stirred overnight at room temperature, the resulting mixture was diluted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification *via* column chromatography (silica gel, CH_2Cl_2 : EtOAc, 7 : 3) provided 39 mg (39%) of **11b** as a semi-solid. ^1H NMR (300 MHz, CD_3OD): δ 1.42 (9H, s, *t*-BuO); 1.58 (4H, m, 4′-H and 5′-H); 2.38~2.81 (2H, m, 2′-H); 2.52 (3H, s, S–CH_3); 3.11 (3H, s, N–CH_3); 3.49 (1H, m, 3′-H); 3.86 (4H, m, 6′-H and 6-H); 4.70 (1H, m, 5-H); 4.95 (2H, m, OCH_2Ph); 5.07 (2H, s, OCH_2Ph); 5.16 (2H, m, OCH_2Ph); 7.34 (15H, m, Ar-H); 7.51 (2H, m, Ar-H); 7.88 (1H, m, Ar-H); 8.21 (2H, m, Ar-H); IR (NaCl, film): 3388, 2974, 1715, 1608, 1538 cm^{-1}. HR-MS (FAB): Calcd for ($C_{49}H_{58}N_8O_{11}S$+H)=967.4024, Found (M+ H)=967.4051.

Cyclization Product **12b**:

To a mixture of **11b** (45 mg, 0.042 mmol, 1.0 eq) in CH_2Cl_2 (0.5 ml) is added TFA (0.5 ml). The resulting mixture was stirred for 15 minutes at room temperature. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on *vacuo* for 2 hours and triturated with dry ether to give a white solid. This white solid was taken into THF (1.0 ml). To this solution was added triethylamine (20 μl, 0.136 mmol, 4.0 eq). After stirring the solution for 10 minutes, the solvent was evaporated and the resulting residue was purified on PTLC (silica gel, CH_2Cl_2 : EtOAc : MeOH, 4 : 1 : 0.5) to give 4 mg (10%) of **12b** as a colorless oil. ^1H NMR (300 MHz, CD_3OD): δ 1.62 (4H, m, 5′-H and 6′-H); 2.56 (2H, m, 2′-H); 3.02 (3H, s, N–CH_3); 3.37 (1H, m, 6′-H); 3.70 (1H, m, 6′-H); 3.93 (3H, m, 3′-H and 6-H); 5.02 (3H, m, 5-H and OCH_2Ph); 5.12 (2H, s, OCH_2Ph); 5.25 (2H, s, OCH_2Ph); 7.32 (18H, m, Ar-H); 8.15 (2H, d, J=7.2 Hz, Ar-H); IR (NaCl, film): 3385, 3263, 3056, 2927, 1720, 1633 cm^{-1}; HR-MS (FAB): Calcd for ($C_{43}H_{46}N_8O_9$+H)=819.3466, Found (M+H)= 819.3436.

3*S*,5′*S*/*R*-3-Amino-6-[(aminoiminomethyl)amino]-*N*-(2-benzoylamino-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-*N*-methyl-hexanamide **13b**:

Prepared from **12b** (3 mg) as described for **13a** to give a 2HCl salt of **13b** (2 mg, 99%) as a colorless amorphous solid. ^1H NMR (300 MHz, D_2O): δ 1.76 (4H, m, 4′-H and 5′-H); 2.85 (1H, m, 2′-H); 3.02 (1H, m, 2′-H); 3.18 (3H, s, N–CH_3); 3.26 (2H, t, J=5.4 Hz, 6′-H); 3.69 (1H, m, 3′-H); 4.05 (2H, m, 6-H); 5.18 (1H, dd, J=8.7 Hz, 5-H); 7.62 (2H, t, J=7.2 Hz, Ar-H); 7.77 (1H, t, J=6.6 Hz, Ar-H), 7.98 (2H, d, J=7.2 Hz, Ar-H); IR (NaCl, film): 3398, 2935, 1732, 1650 cm^{-1}. HR-MS (FAB): Calcd for ($C_{19}H_{28}N_8O_2$+H)=417.2363, Found (M+H)=417.2371.

N-Methylcarbonyl-*N*′-butyloxycarbonyl-*S*-methylisothiourea **8c**:

To a solution of **7** (190 mg, 1.0 mmol, 1.0 eq) in CH_2Cl_2 (5.0 ml) was added methyl chloroformate (170 μl/208 mg, 2.2 mmol, 2.2 eq) and TEA (842 μl, 6.0 mmol, 6.0 eq). The mixture was stirred for 16 hours at room temperature. The resulting mixture was diluted with CH_2Cl_2 (50 ml), washed with brine, dried over Na_2SO_4, filtered and concentrated. Purification *via* column chromatography (silica gel, CH_2Cl_2 : EtOAc, 4 : 1) provided 150 mg (60%) of **8c** as an oil. ^1H NMR (300 MHz, $CDCl_3$ *vs.* TMS): δ 1.50 (9H, s, *t*-BuO); 2.41 (3H, s, S–CH_3); 3.79(3H, s, OCH_3); 11.59 (1H, br, D_2O exchanged, N–H). IR (NaCl, film): 3466, 3187, 1981, 1748, 1659, 1651 cm^{-1}. HR-MS: *Anal* Calcd for ($C_9H_{16}N_2O_4S$+H)=249.0926. Found (M+1)=249.0916.

N-Methylcarbonyl-*S*-methylisothiourea **9c**:

To a mixture of **8c** (140 mg, 0.55 mmol) and anisole (0.1 ml) was added TFA (1.0 ml). The resulting mixture was stirred for 30 minutes at room temperature, evaporated to dryness and put on vacume line for 2 hours, triturated with anhydrous Et_2O to give 140 mg of product as a semi-solid. This crude **9c** was carried on without further purification. ^1H NMR (300 MHz, CD_3OD): δ 2.70 (3H, s, S–CH_3); 3.88 (3H, s, O–CH_3). IR (NaCl, film): 3387, 3283, 3012, 2930, 1672, 1589 cm^{-1}.

Coupling Product **11c**:

To a solution of **10** (320 mg, 0.40 mmol, 1.0 eq), DMAP (146 mg, 1.2 mmol, 3.0 eq) and EDCI·HCl (96 mg, 0.5 mmol, 1.2 eq) in CH_2Cl_2 (2 ml) was added **9c** (131 mg, 0.5 mmol, 1.2 eq). After stirring overnight at room temperature, the resulting mixture was diluted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification *via* column chromatography (silica gel, CH_2Cl_2 : EtOAc, 7 : 3) provided 141 mg (38%) of **11c** as a semi-solid. ^1H NMR (300 MHz, CD_3OD): δ 1.44 (9H, s, *t*-BuO); 1.63 (4H, m, 4′-H and 5′-H); 2.31(3H, s, S–CH_3); 2.60 (2H, m, 2′-H); 3.04 (3H, s, N–CH_3); 3.42 (1H, m, 6′-H); 3.66/3.67 (3H, s, OCH_3); 3.70 (1H, m, 6′-H); 3.93 (3H, m, 3′-H and 6-H); 4.67 (1H, m, 5-H); 5.01 (2H, s, OCH_2Ph); 5.10 (2H, s, OCH_2Ph); 5.24 (2H, s, OCH_2Ph); 7.31 (15H, m, Ar-H); IR (NaCl, CH_2Cl_2): 3389, 2959, 1715 cm^{-1}. HR-MS (FAB): Calcd for ($C_{44}H_{56}N_8O_{12}S$+H) =921.3817. Found (M+H)=921.3834.

Cyclization Product **12c**:

To a mixture of **11c** (46 mg, 0.05 mmol, 1.0 eq) in CH_2Cl_2 (0.5 ml) was added TFA (0.5 ml). The resulting mixture was stirred for 15 minutes. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on *vacuo* for 2 hours and triturated with dry ether to give a white solid. This white solid was dissolved in THF (1.0 ml). To this solution was added triethylamine (15 μl, 0.1 mmol, 2.0 eq). After stirring for 10 minutes, the solvent was evaporated and the resulting residue was purified on PTLC (silica gel, CH_2Cl_2 : EtOAc : MeOH, 4 : 1 : 0.5) to give 9 mg (22%) of **12c** as a colorless oil. ^1H NMR (300 MHz, CD_3OD): δ 1.60 (4H, m, 4′-H and 5′-H); 2.48 (1H, m, 2′-H); 2.57 (1H, m, 2′-H); 2.87/2.93 (3H, s, N–CH_3); 3.02 (1H, m, 6′-H); 3.27 (1H, m, 6′-H); 3.31 (3H, s, O–CH_3); 3.94 (3H, m, 3′-H and 6-H); 4.82 (1H, m, 5-H); 5.02 (2H, s, OCH_2Ph); 5.11 (2H, s, OCH_2Ph); 5.25 (2H, s, OCH_2Ph); 7.30 (15H, m, Ar-H); IR (NaCl, film): 3377, 2917, 1722, 1642 cm^{-1}. HR-MS (FAB): Calcd for ($C_{38}H_{44}N_8O_{10}$+H)=773.3266, Found (M+H)=773.3259.

3*S*,5′*S*/*R*-3-Amino-6-[(aminoiminomethyl)amino]-*N*-(2-methoxycarbonylamino-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-*N*-methyl-hexanamide **13c**:

Prepared from **12c** (9 mg) as described for **13a** to give a 2HCl salt of **13c** (5 mg, 97%) as a white amorphous solid. ^1H NMR (300 MHz, D_2O): δ 1.76 (4H, m, 4′-H and 5′-H); 2.85 (1H, m, 2′-H); 3.01 (1H, m, 2′-H); 3.15 (3H, s, N–CH_3); 3.26 (2H, t, J=6 Hz, 6′-H); 3.69 (1H, m, 3′-H); 3.86 (3H, s, OCH_3); 3.98 (2H, m, 6-H); 5.13 (1H, m, 5-H); IR (KBr, pellet): 3430, 3379, 2948, 1762, 1642 cm^{-1}. HR-MS (FAB): Calcd for ($C_{14}H_{26}N_8O_4$+H)=371.2155, Found (M+H)=371.2170.

N-Methylsulfonyl-*N*′-butyloxycarbonyl-*S*-methylisothiourea **8d**:

To a solution of **7** (380 mg, 2.0 mmol, 1.0 eq) in CH_2Cl_2 (5.0 ml) was added CH_3SO_2Cl (310 μl/458 mg, 2.0 mmol, 2.0 eq) and TEA (842 μl, 6.0 mmol, 3.0 eq). The mixture was stirred for 2 hours at room temperature. The resulting mixture was diluted with CH_2Cl_2 (50 ml), washed with brine, dried (Na_2SO_4), filtered and concentrated. Purification *via* column chromatography (silica gel, CH_2Cl_2 : EtOAc, 4 : 1) provided 500 mg (93%) of **8d** as a yellow oil. ^1H NMR (300 MHz, $CDCl_3$ *vs.* TMS): δ 1.50 (9H, s, *t*-BuO); 2.34 (3H, s, S–CH_3); 3.09 (3H, s, SO_2CH_3); 10.05 (1H, br, D_2O exchanged, N–H). IR (NaCl, film): 3242, 2981, 2934, 1752, 1572 cm^{-1}. HR-MS: *Anal* Calcd for ($C_8H_{16}N_2O_4S_2$+H)=269.0663. Found (M+1)=269.0623

N-Methylsulfonyl-*S*-methylisothiourea **9d**:

To a mixture of **8e** (300 mg, 1.12 mmol) and anisole (0.1 ml) was added TFA (1.0 ml). The resulting mixture was stirred for 30 minutes at room temperature, evaporated to dryness and put on vacuum line for 2 hours, triturated with anhydrous Et_2O to give 300 mg of **9d** as a semi-solid. This crude product was carried on without further purification. ^1H NMR (300 MHz, CD_3OD): δ 2.42 (3H, s, SCH_3); 3.05 (3H, s, SO_2CH_3). IR (NaCl, film): 3405, 3306, 3018, 2925, 1622, 1540 cm^{-1}.

Coupling Product **11d**:

To a solution of **10** (237 mg, 0.30 mmol, 1.0 eq), DMAP (110 mg, 0.9 mmol, 3.0 eq) and EDCI·HCl (61 mg, 0.36 mmol, 1.2 eq) in CH_2Cl_2 (2 ml) was added **9d** (102 mg, 0.5 mmol, 1.2 eq). After stirred overnight at room temperature, the resulting mixture was diluted with

CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification *via* column chromatography (silica gel, CH_2Cl_2 : EtOAc, 7 : 3) provided 97 mg (34%) of **11d** as a semi-solid. ^1H NMR (300 MHz, CD_3OD): δ 1.39 (9H, s, *t*-BuO); 1.64 (4H, m, 4′-H and 5′-H); 2.29 (3H, s, SCH_3); 2.51 ~ 2.76 (2H, m, 2′-H); 3.00 (6H, s, NCH_3, SO_2CH_3); 3.40 (1H, m, 6′-H); 3.64 (1H, m, 6′-H); 3.93 (3H, m, 3′-H, 6-H); 4.55 (1H, m, 5-H); 5.01 (2H, s, OCH_2Ph); 5.11 (2H, s, OCH_2Ph); 5.24 (2H, s, OCH_2Ph); 7.29 (15H, m, Ar-H); IR (NaCl, film): 3388, 2976, 2498, 1714, 1688 cm^{-1}. HR-MS (FAB): Calcd for ($C_{43}H_{56}N_8O_{12}S_2$ + H) = 941.3537, Found (M + H) = 941.3533.

Cyclization Product **12d**:

To a mixture of **11d** (38 mg, 0.04 mmol, 1.0 eq) in CH_2Cl_2 (1 ml) was added anisole (10 μl) and TFA (1 ml). The resulting mixture was stirred for 15 minutes. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on *vacuo* for 2 hours and triturated with dry ether to give a white solid. This white solid was taken into THF (1.0 ml). To this solution was added triethylamine (22 μl, 0.16 mmol, 4.0 eq). After stirring the solution for 10 minutes, the solvent was evaporated and the resulting residue was purified on PTLC (silica gel, CH_2Cl_2 : EtOAc : MeOH, 4 : 1 : 0.5) to give 28 mg (88%) of **12d** as a colorless oil.

^1H NMR (300 MHz, $CD_3OD/CDCl_3$ *vs.* TMS): δ 1.50 (2H, m, 5′-H); 1.63 (2H, m, 6′-H); 2.52 (2H, m, 2′-H); 2.95 (3H, s, NCH_3); 2.97 (3H, s, SO_2CH_3); 3.25 (1H, m, 6′-H); 3.57 (1H, m, 6′-H); 3.90 (3H, m, 3′-H, 6-H); 4.80 (1H, m, 5-H); 5.02 (2H, m, OCH_2Ph); 5.09 (2H, m, OCH_2Ph); 5.22 (2H, s, OCH_2Ph); 7.29 (15H, m, Ar-H); IR (NaCl, film): 3392, 3286, 2940, 1846, 1716, 1506 cm^{-1}; HR-MS (FAB): Calcd for ($C_{37}H_{44}N_8O_{10}S$ + H) = 793.2979, Found (M + H) = 793.3012.

3*S*,5′*S*/*R*-3-Amino-6-[(aminoiminomethyl)amino]-*N*-(2-methylsulfonylamino-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-*N*-methyl-hexanamide **13d**:

Prepared from **12d** (28 mg) as described for **13a** to give a 2HCl salt of **13d** (16 mg, 99%) as a colorless semi-solid. ^1H NMR (300 MHz, D_2O): δ 1.76 (4H, m, 4′-H, 5′-H); 2.84 (1H, m, 2′-H); 3.00 (1H, m, 2′-H); 3.12 (3H, s, N–CH_3); 3.13 (3H, s, SO_2CH_3); 3.26 (2H, t, J = 5.7 Hz, 6′-H); 3.69 (1H, m, 3′-H); 3.76 (1H, m, 6-H); 3.86 (1H, m, 6-H); 5.01 (1H, m, 5-H); IR (KBr pellet): 3411, 3156, 2933, 1733, 1639 cm^{-1}; HR-MS (FAB): Calcd for ($C_{13}H_{26}N_8O_4$ + H) = 391.1876, Found (M + H) = 391.1885.

S-Methylisothiourea **11e**:

To a solution of **10** (120 mg, 0.15 mmol, 1.0 eq), DMAP (37 mg, 0.3 mmol, 2.0 eq) and EDCI·HCl (32 mg, 0.16 mmol, 1.1 eq) in CH_2Cl_2 (0.5 ml) was added **9e** (50 mg, 0.23 mmol, 1.5 eq). The resulting mixture was stirred overnight at room temperature. Then, diluted with CH_2Cl_2 and washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification *via* column chromatography (silica gel, CH_2Cl_2 : EtOAc, 7 : 3) provided 70 mg (52%) of **11e** as a semi-solid. ^1H NMR (300 MHz, CD_3OD): δ 1.40 (9H, s, *t*-BuO); 1.61 (4H, m, 4′-H, 5′-H); 2.32 (3H, s, SCH_3); 2.51 (1H, m, 2′-H); 2.77 (1H, m, 2′-H); 3.02/3.04 (3H, s, NCH_3); 3.40 (1H, m, 6′-H); 3.68 (1H, m, 6′-H); 3.91 (3H, m, 3′-H, 6-H); 4.61 (1/2H, m, 5-H); 4.74 (1/2H, m, 5-H); 4.99/5.00 (2H, s, OCH_2Ph); 5.08 (4H, s, OCH_2Ph); 5.21 (2H, s, OCH_2Ph); 7.27 (20H, m, Ar-H). IR (NaCl, film): 3388, 2976, 1715, 1650, 1609 cm^{-1}. HR-MS (FAB): Calcd for ($C_{50}H_{60}N_8O_{12}S$ + H) = 997.4146, Found (M + H) = 997.4111

Cyclization Product **12e**:

To a mixture of **11e** (40 mg, 0.040 mmol, 1.0 eq) and anisole (20 μl) in CH_2Cl_2 (1.0 ml) was added TFA (1.0 ml). The resulting mixture was stirred for 20 minutes at 0°C. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on *vacuo* for 2 hours and triturated with ethyl ether to give a white solid. This white solid was dissolved in CH_2Cl_2 (1.0 ml). To this solution was added triethylamine (12 μl, 0.04 mmol, 2.0 eq). After stirring the solution for 10 minutes, the solvent was evaporated. Separation *via* PTLC (silica gel, CH_2Cl_2 : EtOAc : MeOH, 4 : 1 : 0.5) provided 17 mg (50%) of **12e** as a colorless oil. ^1H NMR (300 MHz, CD_3OD): δ 1.52 (4H, m, 4′-H, 5′-H); 2.54 (2H, m, 2′-H); 2.98 (3H, s, NCH_3); 3.56 (2H, m, 6′-H); 3.92 (3H, m, 3′-H, 6-H); 5.05 (3H, m, 5-H, OCH_2Ph); 5.11 (2H, s, OCH_2Ph); 5.15 (2H, s, OCH_2Ph); 5.25 (2H, s, OCH_2Ph); 7.31 (20H, m, Ar-H). IR (NaCl, film): 3384, 3272, 2925, 1722, 1645 cm^{-1}. HR-MS (FAB): Calcd for ($C_{44}H_{48}N_8O_{10}$ + H) = 849.3571, Found (M + H) = 849.3571

3*S*,5′*S*/*R*-3-Amino-6-[(aminoiminomethyl)amino]-*N*-(2-amino-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-*N*-methyl-hexanamide **13e**:

Prepared from **12e** (12 mg) as described for **13a** to give a 3HCl salt of **13e** (6 mg, 99% yield) as an amorphous solid. ^1H NMR (300 MHz, D_2O *vs.* DOH): δ 1.76 (4H, m, 4′-H, 5′-H); 2.83 (1H, m, 2′-H); 3.01 (1H, m, 2′-H);

3.13 (3H, s, NCH_3); 3.26 (2H, t, J=5.1 Hz, 6'-H); 3.69 (1H, m, 3'-H); 3.78 (1H, m, 6-H); 3.87 (1H, m, 6-H); 5.06 (1H, m, 5-H); IR (KBr pellet): 3367, 3167, 2922, 1728, 1711, 1650 cm^{-1}. HR-MS (FAB): Calcd for ($C_{12}H_{24}N_8O_2$+H)=313.2094, Found (M+H)=313.2100.

Peptide **16**:

To a mixture of the acid **14** (530 mg, 2.0 mmol, 1.0 eq) and NMM (286 μl, 2.6 mmol, 1.3 eq) in CH_2Cl_2 (2 ml) was added BOP–Cl (664 mg, 2.6 mmol, 1.3 eq) at 0°C, and amine **15** (970 mg, 3.19 mmol, 1.26 eq) in CH_2Cl_2 (3 ml) at 10 minutes later. The resulting mixture was stirred overnight, diluted with CH_2Cl_2 (200 ml), washed with brine, dried over anhydrous Na_2SO_4, and concentrated. Purification *via* column chromatography (silica gel, methylene chloride : EtOAc, 8 : 2) provided 462 mg (42%) of **16** as an oil. ^{1}H NMR (300 MHz, CD_3OD): δ 1.10 (2H, m, 4'-H); 1.37 (4H, m, 3'-H, 5'-H); 1.47 (9H, s, t-BuO); 2.22 (2H, t, J=7.2 Hz, 2'-H); 2.95 (2H, m, 6'-H); 2.98 (3H, s, NCH_3); 4.09 (2H, m, 6-H); 5.04 (2H, s, OCH_2Ph); 5.26 (1H, dd, J=10.2, 4.5 Hz, 5-H); 7.32 (5H, m, CBz–C_6H_5); 7.75 (4H, m, pht–C_6H_4); IR (NaCl, film): 3344, 2935, 1774, 1745, 1650 cm^{-1}. HR-MS (FAB): Calcd for ($C_{30}H_{37}N_3O_7$+H)=552.2710, Found (M+H)=552.2711.

Peptide **18**:

To a solution of **16** (280 mg, 0.51 mmol, 1.0 eq) in THF (10 ml) was added $PdCl_2$ (50 mg). The reaction flask was charged with H_2 from a balloon and the mixture was hydrogenated at 1 atm of H_2 for 2.5 hours. The mixture was then purged with nitrogen, and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo* to give a crude amine as a yellowish solid.

To a solution of the crude amine in CH_2Cl_2 (6 ml) was added *N,N'*-diCBz-*S*-methylthiourea **17** (230 mg, 1.02 mmol, 2.0 eq) and TEA (286 μl, 2.04 mmol, 4.0 eq). The mixture was stirred for 2 hours at room temperature, concentrated and purified on column chromatography (silica gel, CH_2Cl_2 : EtOAc, 8 : 2) to give 18 mg (73%) of **18** as a semi-solid. ^{1}H NMR (300 MHz, CD_3OD): δ 1.12 (2H, m, 4'-H); 1.36 (4H, m, 3'-H, 5'-H); 1.46 (9H, s, t-BuO); 2.23 (2H, t, J=7.2 Hz, 2'-H); 2.96 (3H, s, N–CH_3); 3.23 (2H, m, 6'-H); 4.09 (2H, m, 6-H); 5.10 (2H, s, OCH_2Ph); 5.22 (2H, m, OCH_2Ph); 5.25 (1H, m, 5-H); 7.36 (10H, m, CBz–C_6H_5); 7.72 (2H, m, pht–C_6H_4), 7.80 (2H, m, pht–C_6H_4); IR (NaCl, film): 3339, 2937, 1773, 1718, 1638 cm^{-1}. HR-MS (FAB): Calcd for ($C_{39}H_{45}N_5O_9$+H)=728.3295, Found (M+H)=728.3272.

Acid **19**:

To a solution of **18** (200 mg, 0.275 mmol, 1.0 eq) in CH_2Cl_2 (1.0 ml) was added 2.0 M methylamine/CH_3OH (1.5 ml). The mixture was stirred for 7 minutes at room temperature, concentrated and separated on column chromatography (silica gel, methylene chloride : EtOAc : MeOH, 4 : 1 : 0.3) to give 183 mg of product as an oil.

Then, this crude product was treated with anisole (0.1 ml) and TFA (1.0 ml) at 0°C. The mixture was stirred for 30 minutes at room temperature, concentrated and triturated in dry ether to give 210 mg of solid. This crude solid was taken into H_2O/dioxane (1 ml, 1 : 1). To this mixture was added BOC-on (221 mg, 0.9 mmol, 3.0 eq) and TEA (421 μl, 3.0 mmol, 10 eq). The mixture was stirred overnight and treated with ethyl acetate/sat. NaH_2PO_4 aqueous solution (100 ml, 1 : 1). The organic phase was separated, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification *via* column chromatography (silica gel, CH_2Cl_2 : MeOH, 9 : 1) provided 70 mg (36%) of **19** as a semi-solid. ^{1}H NMR (300 MHz, CD_3OD): δ 1.38 (9H, s, t-BuO); 1.46 (2H, m, 4'-H); 1.62 (4H, m, 3'-H, 5'-H); 2.18(1H, m, 2'-H); 2.40 (1H, m, 2'-H); 2.89 (3H, m, N–CH_3); 3.19 (1H, m, 6'-H); 3.40 (1H, m, 6'-H); 3.58 (1H, m, 6-H); 4.11 (1H, m, 6'-H); 4.45 (1/2H, m, 5-H); 5.13 (2H, m, OCH_2Ph); 5.23 (2H, m, OCH_2Ph); 5.49 (1/2H, m, 5-H); 7.37 (8H, m, Ar-H); 7.70 (1H, m, Ar-H); 7.78 (1H, m, Ar-H); IR (NaCl, film): 3340, 2936, 1716, 1636, 1624 cm^{-1}. HR-MS (FAB): Calcd for ($C_{32}H_{43}N_5O_9$+Na)=664.2958, Found (M+Na)$^+$=664.2982.

Coupling Product **21**:

To a solution of **19** (70 mg, 0.11 mmol, 1.0 eq), DMAP (40 mg, 0.33 mmol, 3.0 eq) and EDCI·HCl (32 mg, 0.165 mmol, 1.1 eq) in CH_2Cl_2 (0.5 ml) was added **20** (61 mg, 0.17 mmol, 1.5 eq). After stirred overnight at room temperature, the resulting mixture was diluted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification *via* column chromatography (silica gel, CH_2Cl_2 : EtOAc, 7 : 3) provided 25 mg (25%) of **21** as a colorless oil. ^{1}H NMR (300 MHz, CD_3OD): δ 1.42 (9H, s, t-BuO); 1.45 (2H, m, 4'-H); 1.61 (4H, m, 3'-H, 5'-H); 2.35 (3H, s, S–H); 2.50 (2H, m, 2'-H); 3.07/3.17 (3H, s, N–CH_3); 3.56 (2H, m, 6'-H); 3.66 (2H, m, 6-H); 4.53 (1H, m, 5-H); 5.10 (2H, s, OCH_2Ph); 5.15 (2H, s, OCH_2Ph); 5.21 (2H, s, OCH_2Ph); 7.35 (15H, m, Ar-H.). IR (NaCl, film): 3340, 2947, 1728, 1644, 1574 cm^{-1}; HR-MS (FAB): Calcd for ($C_{43}H_{54}N_8O_{11}S$+H)=891.3711, Found (M+H)=891.3755.

5'*S*/*R*-6-[(Aminoiminomethyl)amino]-*N*-(2-[amino-carbonyl]amino-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-*N*-methyl-hexanamide **23**:

To a mixture of **21** (20 mg, 0.022 mmol, 1.0 eq) in CH_2Cl_2 (0.5 ml) was added TFA (0.5 ml). The resulting mixture was stirred for 15 minutes at room temperature. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on *vacuo* for 2 hours and triturated with dry ether to give a white solid. This white solid was taken into THF (1.0 ml). To this solution was added triethylamine (8.0 μl, 0.044 mmol, 2.0 eq). After stirring the solution for 10 minutes, the solvent was evaporated and the resulting residue was purified on PTLC (silica gel, CH_2Cl_2 : EtOAc : MeOH, 4 : 1 : 0.5) to give 7 mg (43%) of cyclic intermediate **22** as a colorless oil. Compound **22** was unstable and was used in next step immediately.

To a solution of **22** (4.5 mg, 0.006 mmol, 1.0 eq) in MeOH (0.5 ml)/CH_2Cl_2 (0.5 ml) was added $PdCl_2$ (4 mg). The reaction flask was charged with H_2 from a balloon and the mixture was hydrogenated at 1 atm of H_2 for 15 minutes. The mixture was then purged with nitrogen and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo* to give a 2HCl salt of **23** (2.5 mg, 99%) as a semi-solid. ^{1}H NMR (300 MHz, D_2O): δ 1.40 (2H, m, 4'-H); 1.62 (4H, m, 3'-H, 5'-H); 2.51 (2H, t, J=7.5 Hz, 2'-H); 3.17 (3H, s, N–CH_3); 3.20 (2H, m, 6'-H); 3.93 (2H, d, J=10.2 Hz, 6-H); 4.97 (1H, t, J=10 Hz, 5-H); IR (KBr, pellet): 3491, 2958, 1720, 1651 cm^{-1}. HR-MS (FAB): Calcd for ($C_{13}H_{24}N_8O_3$ + NH_4^+) = 358.2315, Found $(M+NH_4)^+$ = 358.2334.

N-CBz-β-homoglycine **25**:

To a solution of **24** (1.05 g, 5.0 mmol, 1.0 eq) in THF (30 ml) was added NMM (604 μl, 5.5 mmol, 1.1 eq) and ethyl chloroformate (526 μl, 5.5 mmol, 1.1 eq) at 0°C. The resulting mixture was stirred for 1 hour at 0°C. Then the precipitated amine hydrochloride was rapidly filtered off in the cold. To this clear solution was added CH_2N_2/ether solution (generated from MNNG). The solution was stirred overnight at room temperature and concentrated to give an oily diazoketone.

The oily diazoketone was taken into *t*-BuOH/H_2O (40 ml, 1 : 1), and to this solution was added silver benzoate (500 mg) and triethyl amine (4.0 ml). The resulting mixture was stirred overnight in the dark and then concentrated *in vacuo*. The residue was dissolved in ethyl acetate/sat. NaH_2PO_4 aq. and the organic layer was separated, dried over anhydrous sodium sulfate. After filtration, the solvents were evaporated and the crude product was recrystalyzed in EtOAc/Hexane (1 : 1) to give 1.30 g (58%) of **25** as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 2.49 (2H, t, J=6.9 Hz, –CH_2CO–); 3.36 (2H, m, N–CH_2–); 5.06 (2H, s, OCH_2Ph); 7.32 (5H, m, Ar-H.). IR (NaCl, film): 3332, 3026, 2911, 1694, 1684, 1650, 1538 cm^{-1}. mp: 104~105°C; HR-MS (DCI): Calcd for ($C_{11}H_{13}NO_4$ + H) = 224.0923, Found (M + H) = 224.0928.

Peptide **26**:

To a mixture of the acid **24** (200 mg, 0.9 mmol, 1.0 eq) and NMM (128 μl, 1.17 mmol, 1.3 eq) in CH_2Cl_2 (2 ml) was added BOP–Cl (300 mg, 1.17 mmol, 1.3 eq) at 0°C. The reaction mixture was stirred for 10 minutes at 0°C. Then, to the resulting mixture was added amine **14** (270 mg, 0.89 mmol, 1.0 eq) in CH_2Cl_2 (3 ml). The mixture was stirred overnight at room temperature, diluted with CH_2Cl_2 (200 ml), washed with brine, dried over anhydrous Na_2SO_4. After concentration, the residue was separated on column chromatography (silica gel, eluted with methylene chloride : EtOAc : MeOH, 8 : 2 : 0.02) to give 290 mg (64%) of **26** as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (9H, s, *t*-BuO); 2.39 (2H, m, –CH_2CO–); 2.93 (3H, s, N–CH_3); 3.31 (2H, m, –NCH_2–); 4.14 (2H, m, –CH_2Npht); 5.02 (2H, m, OCH_2Ph); 5.26 (1H, m, $CHCO_2Bu^t$); 5.28 (br, D_2O exchanged, N–H); (7.33, 5H, m, Ar-H.); 7.55 (2H, m, pht-H); 7.78 (2H, pht-H). IR (NaCl, film): 3390, 2978, 1774, 1715, 1650 cm^{-1}. HR-MS (DEI): Calcd for $C_{27}H_{31}N_3O_7$ = 509.2161, Found M^+ = 509.2157.

Acid **27**:

To a solution of **26** (90 mg, 0.18 mmol, 1.0 eq) in MeOH (2.0 ml) was added hydrazine (55 mg, 1.8 mmol, 10 eq). The resulting mixture was stirred for 3 hours, concentrated, and dried on *vacuo* overnight to give a white solid. The white solid was treated with CH_2Cl_2/sat. $NaHCO_3$ (50 ml, 1 : 1). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered and concentrated to give an oily residue. This crude amine was treated with TFA (1.0 ml) and stirred for 2 hours. After evaporation of TFA, the residue was taken into H_2O/*t*-BuOH (1.0 ml, 1 : 1). To this solution were added (t-BOC)$_2$O (90 mg, 0.41 mmol, 2.3 eq) and 2 N NaOH solution (300 μl). The resulting mixture was stirred for 16 hours, diluted with water (20 ml) and extracted with Et_2O. The aqueous layer was acidified to pH 4 by 1 N HCl solution and extracted with CH_2Cl_2 (2 × 30 ml). The CH_2Cl_2 extracts were combined, dried over anhydrous Na_2SO_4, filtered, and concentrated. Purification *via* column chromatography

(silica gel, CH_2Cl_2 : MeOH, 9 : 1) provided 29 mg (38%) of **27** as an oil. ^1H NMR (300 MHz, CD_3OD): δ 1.40 (9H, s, *t*-BuO); 2.55 (2H, m, $-CH_2CO-$); 2.81/2.93 (3H, s, $N-CH_3$); 3.36 (1H, m, CBzN–CH_2-); 3.42 (1H, m, $-CH_2$Npht); 3.60 (1H, m, $-CH_2$Npht); 4.44 (1/2H, m, $-CHCO_2Bu^t$); 4.96 (1/2H, m, $-CHCO_2Bu^t$); 5.06 (2H, s, OCH_2Ph); 7.33 (5H, m, Ar-H). IR (NaCl, film): 3338, 2976, 1697, 1622 cm^{-1}. HR-MS (FAB): Calcd for $(C_{20}H_{29}N_4O_7+H)=424.2084$, Found $(M+H)=424.2087$.

Coupling Product **28**:

To a solution of **27** (43 mg, 0.10 mmol, 1.0 eq), DMAP (15 mg, 0.12 mmol, 1.2 eq) and EDCI·HCl (23 mg, 0.12 mmol, 1.2 eq) in CH_2Cl_2 (0.5 ml) was added **20** (44 mg, 0.12 mmol, 1.2 eq). After stirred overnight at room temperature, the resulting mixture was diluted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. Purification *via* column chromatography (silica gel, CH_2Cl_2 : EtOAc, 7 : 3) provided 26 mg (39%) of **28** as a semi-solid. ^1H NMR (300 MHz, CD_3OD): δ 1.45 (9H, s, *t*-BuO); 2.32/2.36 (3H, s, $S-CH_3$); 2.72 (2H, m, $-CH_2CO-$); 3.07/3.12 (3H, s, $N-CH_3$); 3.42 (2H, m, CBzN–CH_2); 3.63 (2H, m, $-CH_2$–Npht); 4.59 (1H, m, $CHCO_2Bu^t$); 5.03 (2H, m, OCH_2Ph); 5.21 (2H, m, OCH_2Ph); 7.31 (10H, m, Ar-H); IR (NaCl, film): 3356, 2944, 1702, 1646, 1532 cm^{-1}. HR-MS (FAB): Calcd for $(C_{31}H_{40}N_6O_9S+H)=673.2656$, Found $(M+H)=673.2680$.

5′*S*/*R*-3-Amino-*N*-(2-[aminocarbonyl]amino-1,4,5,6-tetrahydro-4-oxo-5-pyrimidinyl)-*N*-methyl-propanamide **30**:

To a mixture of **28** (16 mg, 0.024 mmol, 1.0 eq) in CH_2Cl_2 (0.5 ml) was added TFA (0.5 ml). The resulting mixture was stirred for 15 minutes at room temperature. The TFA was evaporated and coevaporated with CH_2Cl_2 to dryness. The resulting residue was dried on *vacuo* for 2 hours and triturated with dry ether to give a white solid. This white solid was taken into THF (1.0 ml). To this solution was added triethylamine (8.0 μl, 0.048 mmol, 2.0 eq). After stirring the solution for 10 minutes, the solvent was evaporated and the resulting residue was purified on PTLC (silica gel, CH_2Cl_2 : EtOAc : MeOH, 4 : 1 : 0.5) to give 4 mg of cyclic product **29** as a colorless oil. This oily cyclic compound was taken into MeOH (0.5 ml)/CH_2Cl_2 (0.5 ml). To the resulting solution was added $PdCl_2$ (4 mg). The reaction flask was charged with H_2 from a balloon and the mixture was hydrogenated at 1 atm of H_2 for 15 minutes. The mixture was then purged with nitrogen and filtered to remove the catalyst. The filtrate was concentrated and dried *in vacuo*. to give a 2HCl salt of **30** (2 mg, 25%) as a colorless amorphous solid. ^1H NMR (300 MHz, D_2O): δ 2.94 (2H, m, 2′-H); 3.14 (3H, s, $N-CH_3$); 3.28 (2H, t, $J=6.0$ Hz, 3′-H); 3.96 (2H, m, 6-H); 5.11 (1H, dd, $J=11.9$, 8.7 Hz, 5-H); IR (KBr, pellet): 3450, 3198, 2976, 1743, 1620 cm^{-1}. HR-MS (FAB): Calcd for $(C_9H_{16}N_6O_3+H)=257.1283$, Found $(M+H)=257.1351$.

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