**Supplement to:** A. G. Tereshchenkov, A. V. Shishkina, V. N. Tashlitsky, G. A. Korshunova, A. A. Bogdanov, N. V. Sumbatyan, Interaction of Chloramphenicol Tripeptide Analogs with Ribosomes (ISSN 0006-2979, *Biochemistry (Moscow)*, 2016, Vol. 81, No. 4, pp. 392-400)

**Table S1.** Designed chloramphenicol peptide analogs and corresponding ribosome arrest peptide sequences [1]

| Chloramphenicol peptide analog | Arrest sequence | Ribosome arrest peptide (*E. coli*) |
|---|---|---|
| AcMRL-CAM | MTAS**MRL**K | MsrAL |
| | MTHS**MRL**R | ErmDL |
| AcIRA-CAM | FSTPVWISQAQG**IRA**GP | SecM |
| AcIWP-CAM | FQKYG**IWP**PP | WPPP |

**Procedure of synthesis of AcMetArgLeu-dACaegCaeg (IV).** *Bis-$N^6$,5′-O-(4,4′-dimethoxytrityl)-2′-desoxyadenosine (**IX**)*. To 290 mg (1.15 mmol) of 2′-desoxyadenosine in 4.5 ml of absolute pyridine 1.17 g (3.46 mmol) of DMTCl, 5.6 mg (0.046 mmol) of DMAP and 196 μl of DIPEA were added and the mixture was stirred at room temperature for 3 h. The excess of DMTCl was neutralized with methanol (2 ml). Then the mixture was stirred for 15 min and evaporated, the residue was dissolved in 15 ml of $CHCl_3$, and washed with a saturated solution of $NaHCO_3$ (10 ml) and with water (2 × 10 ml). The organic fraction was dried over $Na_2SO_4$ (anhydrous). The desiccant was filtered and the filtrate was evaporated. The crude product was purified by column chromatography on silica gel with the system: concentration gradient of ethanol in $CHCl_3$ from 0 to 5% with the addition of 0.1% of pyridine. Yield of **IX**: 315 mg (32%); T.m. 127-130°C; TLC: $R_f$($CHCl_3$–MeOH, 9 : 1) 0.82, $R_f$($CHCl_3$–MeOH, 95 : 5) 0.42; LC-MS, m/z calculated for $C_{52}H_{50}N_5O_7^+$, 856.4, found 856.8; $^1$H-NMR: ($CDCl_3$, 400 MHz) 8.00 ppm (s, 1H, H-8), 7.89 ppm (s, 1H, H-2), 7.36-7.39 ppm (m, 2H, p-H Phe), 7.21-7.33 ppm (m, 16H, o-H, m-H Phe-OMe), 6.91 ppm (s, 1H, NH), 6.76-6.81 ppm (m, 8H, o-H, m-H Phe), 6.39 ppm (t, $J_t$ = 6.57 Hz, 1H, H-1′), 4.63 ppm (m, 1H, H-3′), 4.11 ppm (m, 1H, H-4′), 3.76 and 3.77 ppm (s, 12H, $OCH_3$), 3.37-3.40 ppm (m, 2H, H-5′), 2.74-2.80 ppm (m, 1H, H-2′a), 2.44-2.50 ppm (ddd, 1H, H-2′b).

Additionally, *$DMT_4dA$* was isolated: yield, 590 mg (35%); TLC: $R_f$($CHCl_3$–MeOH, 9 : 1) 0.92, $R_f$($CHCl_3$–MeOH, 95 : 5) 0.63; $^1$H-NMR: ($CDCl_3$, 400 MHz) 8.01 ppm (s, 1H, H-8), 7.81 (s, 1H, H-2), 6.71-6.84 and 7.14-7.41 ppm (m, 52H, DMT), 6.43 ppm (dd, 1H, H-1′), 4.41 ppm (m, 1H, H-3′), 4.13 ppm (m, 1H, H-4′), 3.75, 3.76, 3.77 and 3.80 ppm (s, 24H, $OCH_3$), 3.21-3.24 ppm (m, 1H, H-5′a), 3.00-3.05 ppm (m, 1H, H-5′b), 2.29 ppm (m, 1H, H-2′a), 2.85 ppm (m, 1H, H-2′b).

*$DMT_2$dA-LeuArg(Pbf)MetAc (**X**)*. To a cooled to 0°C solution of 52 mg (73 mmol) of AcMetArg(Pbf)LeuOH in 220 μl of DMF a solution of 17 mg (82 mmol) of DCC in 100 μl DMF and 10 mg (73 mmol) of HOBt were added with stirring. Stirring was continued for 1 h at 0°C, after which a solution of 50 mg (58 mmol) $DMT_2$dA (**IX**) and 7.1 mg (58 mmol) of DMAP in 250 μl of DMF was added. The reaction mixture was stirred 48 h at room temperature. Then the reaction mixture was diluted with water (3 ml), extracted with $CHCl_3$ (three times 3 ml), washed with saturated $NaHCO_3$ (2 × 3 ml), water (three times 3 ml) and saturated NaCl (3 ml). Then organic layer was dried over anhydrous $Na_2SO_4$, filtered, and evaporated on a rotary evaporator. The product was purified by column chromatography in the solvent system: $CHCl_3$–ethyl acetate–MeOH–pyridine, 6 : 3 : 0.5 : 0.01. Yield of **X**: 25 mg (28%); TLC: $R_f$($CHCl_3$–MeOH, 9 : 1) 0.58, $R_f$($CHCl_3$–ethyl acetate–MeOH, 6 : 3 : 0.5) 0.23; LC-MS, m/z calculated for $C_{84}H_{100}N_{11}O_{14}S_2^+$, 1550.7, found 1551.8.

*dA-LeuArg(Pbf)MetAc (**XI**)*. 1.4 ml of 2.4% (v/v) solution of TFA in $CH_2Cl_2$ was added to a cooled solution of 25 mg (0.016 mmol), $DMT_2$dA-LeuArg(Pbf)MetAc (**X**) in 0.8 ml of dry $CH_2Cl_2$ and a mixture was kept for 2.5 h at 0°C. Then, 1.3 ml of a mixture of pyridine–water (1 : 1, v/v) was added to neutralize TFA. The organic layer was separated, washed with water and evaporated on a rotary evaporator. The resulting oil was purified using column chromatography in the solvent system $CHCl_3$–MeOH, 9 : 1. Yield of **XI**: 11.6 mg (77%); TLC:

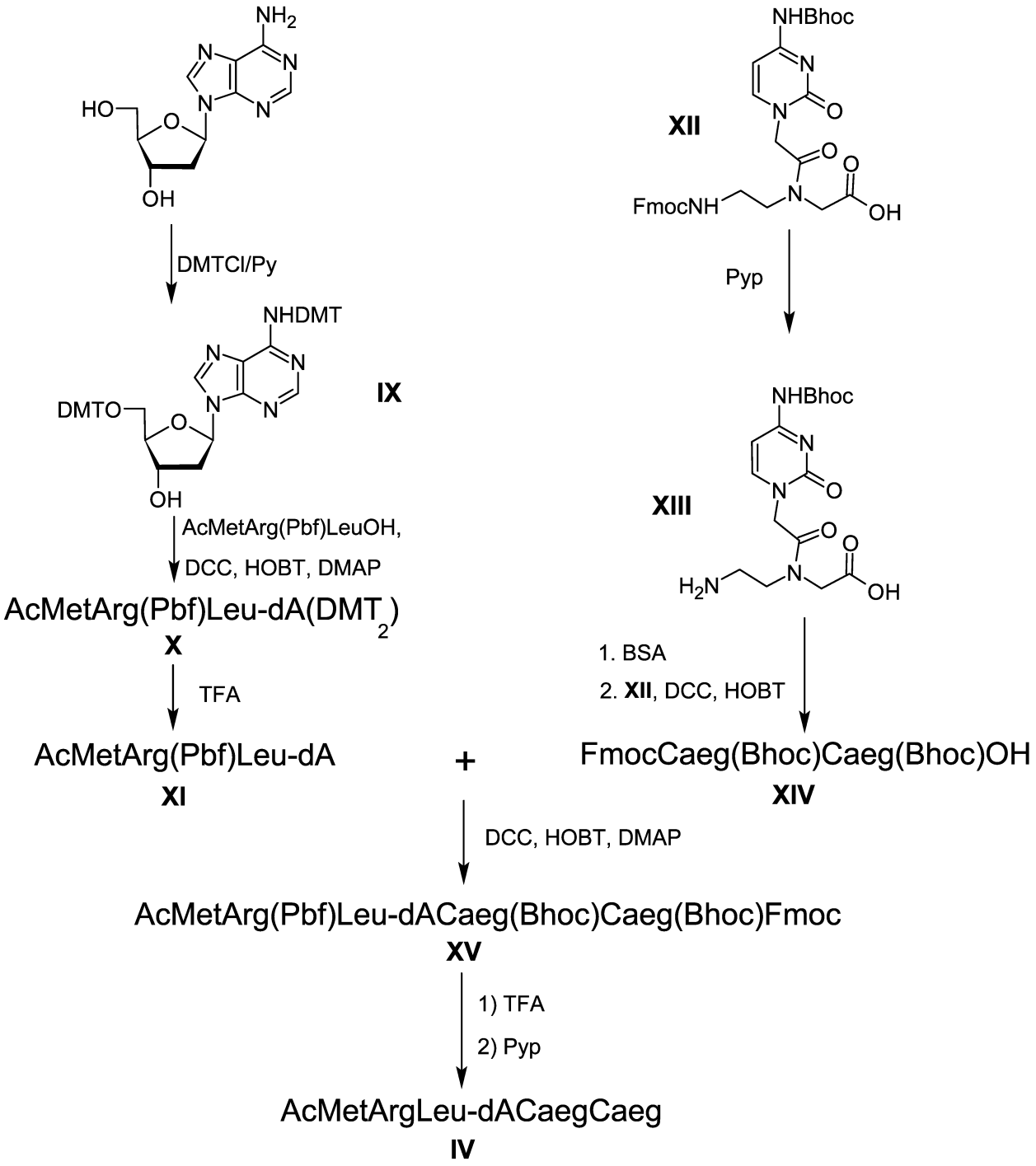


**Fig. S1.** Scheme of synthesis of AcMetArgLeu-dACaegCaeg (**IV**).

$R_f$($CHCl_3$–MeOH, 9 : 1) 0.15, $R_f$($CHCl_3$–MeOH, 3 : 1) 0.82; UV ($H_2O$): $\lambda_{max}$ – 222 and 260 nm; LC-MS, m/z calculated for $C_{42}H_{64}N_{11}O_{10}S_2^+$, 946.4, found 946.9; MALDI-TOF MS, m/z calculated for $C_{42}H_{64}N_{11}O_{10}S_2^+$, 946.4, found 946.4.

*Caeg(Bhoc)OH (**XIII**)*. 100 mg (0.143 mmol) FmocCaeg(Bhoc)OH (**XII**) was dissolved in 1 ml of piperidine and stirred for 1 h at room temperature. Then, the solvent was evaporated on a rotary evaporator and a product was precipitated with ether from $CH_2Cl_2$. The resulting material was used in the subsequent stages of the synthesis without further purification. Yield of **XIII**: 67 mg (99%); TLC: $R_f$($CHCl_3$–water–MeOH, 65 : 4 : 25) 0.85.

*FmocCaeg(Bhoc)Caeg(Bhoc)OH (XIV)*. A mixture of 67 mg (0.140 mmol) Caeg(Bhoc)OH (**XIII**), 96 μl (0.392 mmol) of bis-(N-trimethylsilyl)acetamide and 500 μl of dry $CH_2Cl_2$ was intensively stirred in a sealed flask at room temperature until complete dissolution. At the same time: 28 mg (0.209 mmol) of HOBt and 43 mg (0.209 mmol) of DCC were added with stirring to 98 mg (0.140 mmol) of FmocCaeg(Bhoc)OH (**XII**) in a mixture of 500 μl of $CH_2Cl_2$ and 100 μl of NMP at 0°C. Stirring was conducted for 5 h, after which the first reaction mixture was added with addition of 24 μl of DIPEA and the combined mixture was left overnight at room temperature. Then the mixture was diluted with $CH_2Cl_2$ (5 ml), washed with 1% citric acid solution (3 ml) and water (3 ml). The organic fraction was dried over anhydrous $Na_2SO_4$ and was evaporated on a rotary evaporator. The resulting material was purified by column chromatography in the $CHCl_3$–water–MeOH, 65 : 3 : 25 and dried in a desiccator over $CaCl_2$. For LC-MS analysis, Bhoc protecting groups were removed: 0.5 mg of the substance was treated with 50 μl of TFA for 5 min, evaporated, and dissolved in MeOH. Yield of **XIV**: 16 mg (10%); TLC: $R_f$($CHCl_3$–water–MeOH, 65 : 4 : 25) 0.19, $R_f$($CHCl_3$–19% $NH_4OH$–MeOH, 65 : 4 : 25) 0.09; LC-MS, m/z calculated for $C_{35}H_{39}N_{10}O_9^+$, 743.3, found 743.7.

*FmocCaeg(Bhoc)Caeg(Bhoc)dA-LeuArg(Pbf)MetAc (XV)*. A solution of 2.1 mg (0.015 mmol) HOBt and of 3.8 mg (0.018 mmol) of DCC in 50 ml of DMF was added to a cooled to 0°C solution of 16 mg (0.014 mmol) FmocCaeg(Bhoc)Caeg(Bhoc)OH (**XIV**) in 100 μl DMF. Stirring of the reaction mixture were continued for 5 h at 0°C, then the mixture was left in the refrigerator overnight. Then 3 mg (0.015 mmol) of DCC and 0.8 mg (0.006 mmol) of HOBt in 10 ml DMF were added and the mixture was stirred for 1.5 h at 0°C. Then a solution of 11.6 mg (0.012 mmol) dA-LeuArg(Pbf)MetAc (**XI**) and 1.5 mg (0.012 mmol) of DMAP in 300 μl $CH_2Cl_2$ were added to the reaction mixture. The mixture was stirred at room temperature for 20 h and separated by column chromatography in the $CH_2Cl_2$–EtOH, 2 : 1. For MALDI-TOF MS analysis, Bhoc protecting groups were removed: 0.5 mg of the substance was treated with 50 μl of TFA for 5 min, evaporated, and dissolved in methanol. Yield of **XV**: 20.7 mg (81%); TLC: $R_f$($CHCl_3$–MeOH, 9 : 1) 0.15, $R_f$($CHCl_3$–MeOH, 2 : 1) 0.89; MALDI-TOF MS, m/z calculated for $C_{77}H_{100}N_{21}O_{18}S_2^+$, 1670.7, found 1670.6.

*TFA·CaegCaegdA-LeuArgMetAc (IV)*. To 10.8 mg (5.16 mmol) of FmocCaeg(Bhoc)Caeg(Bhoc)dA-LeuArg(Pbf)MetAc (**XV**), 500 μl of a mixture consisting of 412 μl TFA, 25 μl of phenol, 25 μl *m*-cresol, 25 μl of water and 12.5 mg of DTT was added. The reaction mixture was stirred at room temperature for 2.5 h, evaporated to dryness on a rotary evaporator, methanol was added, and the mixture was evaporated again and the product was precipitated with ether from methanol. The resulting precipitate was separated and 600 μl of piperidine were added. After 1 h stirring at room temperature, the piperidine was evaporated on a rotary evaporator; the resulting oil was dissolved in minimum amount of DMF and precipitated with ether. The precipitate was washed again with ether, and dried in a vacuum desiccator over $CaCl_2$. Yield of **IV**: 3.3 mg (53%); TLC: $R_f$(PrOH–19% $NH_4OH$–water, 14 : 1 : 5) 0.7; UV ($H_2O$): $\lambda_{max}$ – 269 nm; MALDI-TOF MS, m/z calculated for $C_{49}H_{74}N_{21}O_{13}S^+$, 1196.5, found 1196.5.

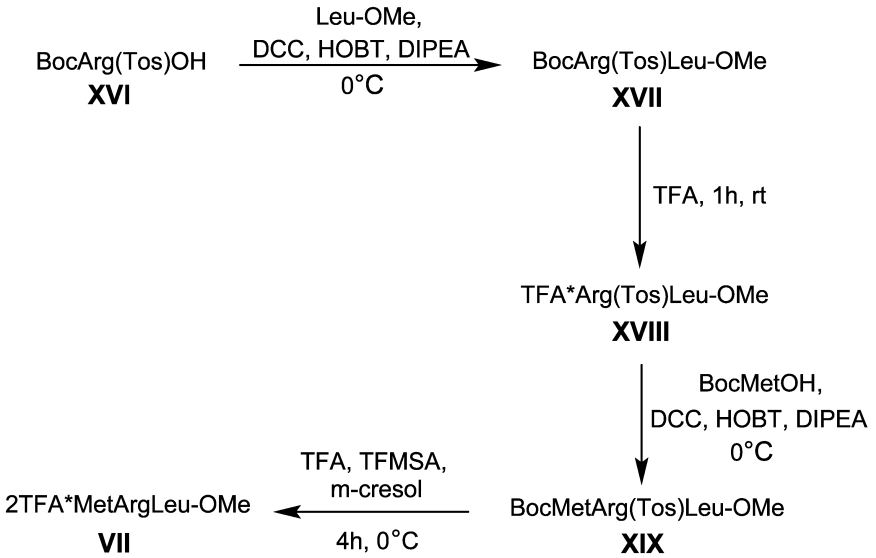


**Fig. S2.** Scheme of synthesis of MetArgLeu-OMe (**VII**).

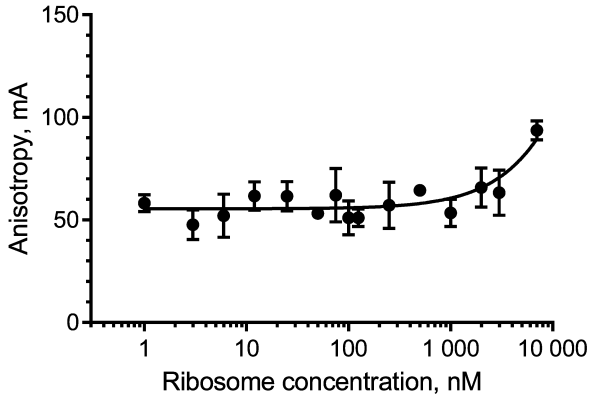


**Fig. S3.** Binding of BODIPY-MetArgLeu-OMe (4 nM) to 70S *E. coli* ribosomes.

**Procedure of synthesis of MetArgLeu-OMe (VII).** *BocArg(Tos)Leu-OMe (XVII)*. To a cooled to 0°C solution of 400 mg (0.796 mmol) of BocArg(Tos)OH·4/5AcOEt·1/5$H_2O$ (**XVI**) and 140 mg (1.035 mmol) HOBt in 1 ml DMF a solution of 213 mg (1.035 mmol) of DCC in 0.5 ml DMF was added with stirring. Stirring was continued for 2 h at 0°C, after which a solution of 152 mg (0.835 mmol) of HCl·Leu-OMe and 135 µl DIPEA in 1 ml DMF was added. The reaction mixture was stirred 72 h at room temperature. After filtration of dicyclohexylurea (DCU) precipitate the reaction mixture was diluted with water (10 ml), extracted with ethyl acetate (three times 5 ml), washed with 0.05 M solution of $H_2SO_4$ (3 × 5 ml), water (5 ml), 5% solution of $NaHCO_3$ (3 × 5 ml) and saturated NaCl (2 ml). Then organic layer was dried over anhydrous $Na_2SO_4$, filtered, and evaporated on a rotary evaporator. The obtained product was precipitated with petroleum ether from ethyl acetate, purified by column chromatography in the solvent system: $CHCl_3$–MeOH, 96 : 4 and dried in a desiccator over $CaCl_2$. Yield of **XVII**: 367 mg (83%); TLC: $R_f$($CHCl_3$–MeOH, 9 : 1) 0.72, $R_f$($CHCl_3$–MeOH, 4 : 1) 0.90, $R_f$($CHCl_3$–MeOH, 96 : 4) 0.13; UV ($H_2O$): $\lambda_{max}$ – 235 nm; T.m. 135-138°C; $[\alpha]_D^{20}$ –24.0 (c = 1.05; MeOH); LC-MS, m/z calculated for $C_{25}H_{42}N_5O_7S^+$, 556.3, found 556.3; $^1$H-NMR: ($CDCl_3$, 400 MHz); δ (ppm) 7.77 (d, $J_d$ = 7.58 Hz, 2H, o-H, -$SO_2$-Phe), 7.25 (d, $J_d$ = 7.96 Hz, 2H, m-H, -$SO_2$-Phe), 6.46 (bs, 2H, NH), 5.53 (bs, 1H, NH), 4.53 (dd, 1H, C(O)-CH($CH_2$)-NH), 4.32 (bs, 1H, C(O)-CH($CH_2$)-NH), 3.69 (s, 3H, $OCH_3$), 3.26 (bs, 2H, $CH_2$-NH), 2.40 (s, 3H, $CH_3$Phe), 2.11 (bs, 1H, NH), 1.85 (bs, 1H, NH), 1.61 (m, 6H, $CH_2$), 1.45 (m, 1H, $CH_2CH(CH_2)_2$), 1.42 (s, 9H, $CH_3$tBu), 0.89 (d, $J_d$ = 5.81 Hz, 6H, $(CH_3)_2$-CH).

*TFA·Arg(Tos)Leu-OMe (XVIII)*. A 317-mg (0.570 mmol) sample of BocArg(Tos)Leu-OMe (**XVII**) was dissolved in 1 ml TFA and stirred for 1 h at room temperature. Then TFA was evaporated to dryness on a rotary evaporator, methanol was added and the mixture was evaporated again. Obtained product was precipitated with ether from methanol and dried in a vacuum desiccator over $CaCl_2$. The resulting material was used in the subsequent stages of the synthesis without further purification. Yield of **XVIII**: 319 mg (98%); TLC: $R_f$($CHCl_3$–MeOH, 9 : 1) 0.16, $R_f$($CHCl_3$–MeOH, 4 : 1) 0.46.

*BocMetArg(Tos)Leu-OMe (XIX)*. To a cooled to 0°C solution of 135 mg (0.543 mmol) of BocMetOH and 110 mg (0.814 mmol) HOBt in 700 µl DMF, a solution of 168 mg (0.814 mmol) of DCC in 350 µl DMF was added with stirring. Stirring was continued for 2 h at 0°C, after which a solution of 319 mg (0.559 mmol) of TFA·Arg(Tos)Leu-OMe (**XVIII**) and 100 µl DIPEA in 700 µl DMF was added. The reaction mixture was stirred 72 h at room temperature. After filtration of DCU precipitate the reaction mixture was diluted with water (10 ml), extracted with ethyl acetate (three times 5 ml), washed with 0.05 M solution of $H_2SO_4$ (3 × 5 ml), water (5 ml), 5% solution of $NaHCO_3$ (3 × 5 ml) and saturated NaCl (2 ml). Then organic layer was dried over anhydrous $Na_2SO_4$, filtered, and evaporated on a rotary evaporator. The obtained product was precipitated with petroleum ether from ethyl acetate, purified by column chromatography in the solvent system $CHCl_3$–MeOH (97 : 7) and dried in a desiccator over $CaCl_2$. Yield of **XIX**: 272 mg (73%); TLC: $R_f$($CHCl_3$–MeOH, 9 : 1) 0.76, $R_f$($CHCl_3$–MeOH, 4 : 1) 0.55; UV ($H_2O$): $\lambda_{max}$ – 232 nm; T.m. 95-98°C; LC-MS, m/z calculated for $C_{30}H_{51}N_6O_8S_2^+$, 687.3, found 687.3.

*2TFA·MetArgLeu-OMe (VII)*. To 50 mg (73 µmol) of BocMetArg(Tos)Leu-OMe (**XIX**), a mixture consisting of 140 µl TFA, 51 µl $CF_3SO_3H$ and 31 µl *m*-cresol was added at 0°C. After 4 h stirring at 0°C, the reaction mixture was evaporated on a rotary evaporator. The resulting oil was dissolved in minimum amount of water and precipitated with ether. The precipitate was washed again with ether, and dried in a vacuum desiccator over $CaCl_2$. Yield of **VII**: 20 mg (42%); TLC: $R_f$($CHCl_3$–MeOH–19% $NH_4OH$–$CH_3COOH$, 65 : 25 : 3 : 1) 0.06, $R_f$($CHCl_3$–MeOH–$H_2O$, 10 : 10 : 1) 0.09; AAA: Arg 1.00 (1). Leu 1.00 (1). Met 0.60 (1); MALDI-TOF MS, m/z calculated for $C_{18}H_{37}N_6O_4S^+$, 433.3, found 433.3.

## REFERENCES

1. Ito, K., and Chiba, S. (2013) Arrest peptides: *cis*-acting modulators of translation, *Annu. Rev. Biochem.*, **82**, 171-202.