Supporting Information for:

3-Substituted Imidazo[1,2-*d*]-1,2,4-Thiadiazoles: A Novel Class of Factor XIIIa Inhibitors

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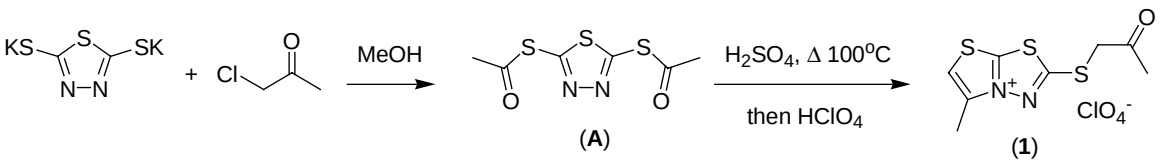
*Synthesis of compound **1**:*[26]



A mixture of 2,5-dimercapto-1,3,4-thiadiazole, dipotassium salt (2.26g, 10 mmol) and chloroacetone (1.6 mL, 20 mmol) in methanol (50 mL) was stirred at room temperature for 2 h. The precipitated solid was collected by suction filtration. The mother liquor was cooled in an ice-bath for 2h as more solid separated. The combined solid fractions weighed 2.6g. The latter was taken up in dichloromethane. The organic layer was washed with water, dried over sodium sulfate, filtered and evaporated to dryness. The solid white product **A** (1.92g) melted at 89-92°C [Lit.[26] 90-91°C]. ^{1}H-NMR ($CDCl_3$) δ ppm: 2.36 (s, 6H, $2CH_3$) and 4.21 (s, 4H, $2CH_2$); ^{13}C-NMR ($CDCl_3$) δ ppm: 200.6, 164.2, 43.8 and 28.9; MS m/z 285 (M^++23), 263 (M^++1), 207, 163, 133, 89.

Compound **A** (1.31g, 5 mmol) in 10 mL conc. sulfuric acid was heated in an oil bath at 100°C for 90 min. The mixture was then poured onto 20g of ice. To this was added 4 mL of a 70% perchloric acid solution. The mixture was cooled in ice. The brown greenish solid was collected by suction filtration. The solid was dissolved in warm water. Celite and activated charcoal were added. The mixture was filtered over a pad of celite and the cake was thoroughly washed with warm water. The filtrate was evaporated to dryness, and the residue was suspended in warm EtOH for 1h. On cooling to RT, the white solid product **1** (190 mg) was collected by suction filtration. The mother liquor was concentrated to half volume, and the precipitated solid was again collected (120 mg). No further attempt was made to recover more product from the mother liquor. M.p. shrunk at 155°C and melted at 195-199°C; ^{1}H-NMR (DMSO-d_6) δ ppm: 7.94 (s, 1H), 4.53 (s, 2H), 2.58 (s, 3H) and 2.33 (s, 3H); ^{13}C-NMR (DMSO-d_6) δ ppm: 201.0, 173.8, 162.8, 137.0, 122.4, 44.3, 28.8, and 12.5; MS m/z 245 ($C_8H_9N_2OS_3^+$), 157, 130, 89.

*Synthesis of N-imidazo[1,2-d][1,2,4]thiadiazol-3-ylhexane-1,6-diamine (**4**)*:

To 3-[(4-methylphenyl)sulfonyl]imidazo[1,2-*d*][1,2,4]thiadiazole (**3**, 14.0 g, 0.05 mol) dissolved in DMF (400 mL) was added 1,6-hexanediamine (29.0 g, 0.25 mol) all at once, followed by triethylamine (14.0 mL, 0.1 mol). The resulting mixture was stirred at room temperature (RT) overnight. Volatile materials were removed *in vacuo* and the residue was partitioned between water (75 mL) and dichloromethane (200 mL). The aqueous layer was extracted with CH_2Cl_2 (7× 200 mL portions). The combined organic fractions was dried over sodium sulfate, then filtered and evaporated to dryness. Purification of the residue by column chromatography using a mixture of solvents (gradient of 10% MeOH in CH_2Cl_2, followed by 20% MeOH in CH_2Cl_2, and finally 8% aqueous NH_3 in 20% MeOH/CH_2Cl_2) afforded the title compound as a light yellow oil. On trituration in a mixture of hexane and ethyl acetate, a white solid was obtained (6.7g, 56%). M.p. 81-82ºC; ^{1}H-NMR (CD_3OD) δ ppm: 7.20 (s, 1H), 7.32 (s, 1H), 3.44 (t, J = 7.0 Hz, 2H), 2.65 (t, J = 7.0 Hz, 2H), 1.69-1.77 (m, 2H) and 1.37-1.55 (m, 6H).

*N-[6-(Imidazo[1,2-d][1,2,4]thiadiazol-3-ylamino)hexyl]benzenesulfonamide (**5**):*

^{1}H-NMR ($CDCl_3$ + CD_3OD) δ ppm: 7.84-7.82 (d, J=8.0 Hz, 2H), 7.72 (br.s, 1H), 7.58-7.48 (m, 3H), 7.32 (br.s, 1H), 3.43-3.39 (t, J=6.9 Hz, 2H, NHC*H*$_2$), 2.91-2.88 (t, J=6.7 Hz, 2H, SO_2NC*H*$_2$), 1.66-1.63 (m, 2H), 1.49-1.45 (m, 2H) and 1.36-1.35 (m, 4H); MS m/z 380(M^++1), 382, 240.

*N-[6-(Imidazo[1,2-d][1,2,4]thiadiazol-3-ylamino)hexyl]-2-methylbenzenesulfonamide (**6**):*

^{1}H-NMR (DMSO-D_6) δ ppm: 7.91-7.89 (m, 2H), 7.60-7.57 (m, 2H), 7.50-7.43 (m, 4H), 3.35-3.30 (m, 2H), 2.73-2.72 (m, 2H), 2.38 (s, 3H, CH_3), 1.56-1.55 (m, 2H), 1.37-1.35 (m, 2H) and 1.25-1.24 (m, 4H); MS m/z 394(M^++1), 296(100%), 254, 184.

*N-[6-(Imidazo[1,2-d][1,2,4]thiadiazol-3-ylamino)hexyl]-4-methylbenzenesulfonamide (**7**)*:

^{1}H-NMR ($CDCl_3$ + CD_3OD) δ ppm: 7.70-7.68 (m, 3H), 7.29-7.27 (m, 3H), 3.41-3.38 (t, J=6.9 Hz, 2H), 2.87-2.84 (t, J=6.6 Hz, 2H), 2.39 (s, 3H, CH_3), 1.65-1.61 (m, 2H), 1.47-1.44 (m, 2H) and 1.35-1.34 (m, 4H); MS m/z 394(M^++1), 296(100%), 254, 184.

*N-[6-(Imidazo[1,2-d][1,2,4]thiadiazol-3-ylamino)hexyl]-2-nitrobenzenesulfonamide (**8**):* Yield: 70.4%; mp 113.7-114.6ºC; ^{1}H-NMR (DMSO-d_6) δ ppm: 8.09-8.06 (t, J=7.6 Hz, 1H, NH), 8.02-7.94 (m, 2H), 7.92-7.89 (t, J=7.6 Hz, 1H, NH), 7.87-7.81 (m, 3H), 7.31 (d, J=2.4 Hz, 1H), 3.33-3.26 (q, J=8.6 Hz, 2H), 2.93-2.87 (q, J=8.8 Hz, 2H), 1.57-1.53 (quint, J=9.0 Hz, 2H), 1.45-1.40 (quint, J=8.8 Hz, 2H) and 1.28-1.27 (m, 4H); ^{13}C-NMR (DMSO-d_6) δ ppm: 156.8, 147.7, 144.2, 136.8, 133.9, 132.8, 132.5, 129.4, 124.3, 111.2, 42.6, 41.6, 29.0, 28.4, 25.9 and 25.6; MS m/z 447.2 (M^++23), 425.2 (M^++1), 327(100%), 285, 215, 185.

*N-[6-(Imidazo[1,2-d][1,2,4]thiadiazol-3-ylamino)hexyl]-3-nitrobenzenesulfonamide (**9**)*: ^{1}H-NMR (DMSO-D_6) δ ppm: 8.52-8.51 (m, 1H), 8.49-8.45 (m, 1H), 8.23-8.20 (m, 1H), 7.98-7.95 (t, J=6.8 Hz, 1H, NH), 7.93-7.88 (m, 2H), 7.85-7.84 (d, J=2.4 Hz, 1H), 7.31-7.30 (d, J=2.0 Hz, 1H), 3.33-3.17 (q, J=8.8 Hz, 2H), 2.82-2.76 (q, J=8.0 Hz, 2H), 1.57-1.53 (quint, J=9.2 Hz, 2H), 1.41-1.37 (quint, J=8.6 Hz, 2H) and 1.27-1.25 (m, 4H); MS m/z 425(M^++1), 327, 285, 215.

*N-[6-(Imidazo[1,2-d][1,2,4]thiadiazol-3-ylamino)hexyl]-4-nitrobenzenesulfonamide (**10**)*:

^{1}H-NMR (DMSO-D_6) δ ppm: 8.44-8.41 (d, J=2.4 Hz, 1H), 8.41 (d, J=2.4 Hz, 1H), 8.06-8.03 (m, 2H), 8.00-7.96 (t, J=7.5 Hz, 1H, NH), 7.92-7.88 (t, J=7.2 Hz, 1H, NH), 7.85-7.84 (d, J=2 Hz, 1H), 7.31-7.30 (d, J=1.6 Hz, 1H), 3.32-3.26 (q, J=8.6 Hz, 2H), 2.84-2.77 (q, J=8.6 Hz, 2H), 1.58-1.53 (quint, J=9.0 Hz, 2H), 1.41-1.37 (quint, J=8.6 Hz, 2H) and 1.28-1.27 (m, 4H); MS m/z 425(M^++1), 327(100%), 285, 215.

*Synthesis of tert-butyl [6-(imidazo[1,2-d][1,2,4]thiadiazol-3-ylamino)hexyl][(2-nitrophenyl)sulfonyl]carbamate (**13**)*:

A mixture of *N*-[6-(imidazo[1,2-*d*][1,2,4]thiadiazol-3-ylamino)hexyl]-2-nitrobenzene sulfonamide (**8**, 480 mg, 1.14 mmol), di-*tert*-butyl dicarbonate (375 mg, 1.70 mmol) and 4-dimethylaminopyridine (DMAP, 75 mg, 0.61 mmol) in acetonitrile (40 mL) was stirred at ambient temperature for 16 h. Volatile materials were removed *in vacuo* and the residue was partitioned between ethyl acetate and a solution of 10% citric acid. The organic fraction was collected, washed with water, then dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by column chromatography on silica gel using a solvent mixture of 4% MeOH in CH_2Cl_2 as eluent, thereby affording the title compound **13** (500 mg) as a colorless oil. Yield: 83.6%; ^{1}H-NMR ($CDCl_3$) δ ppm: 8.25-8.35 (m, 1H), 7.77-7.80 (m, 3H), 7.46 (s, 1H), 7.32 (s, 1H), 5.73 (br. t, 1H, N*H*), 3.80 (t, J = 6.9 hz, 2H), 3.52 (m, 2H), 1.63-1.80 (m, 4H), 1.40-1.50 (m, 4H) and 1.39 (s, 9H); ^{13}C-NMR ($CDCl_3$) δ ppm: 159.1, 150.5, 147.7, 143.9, 137.4, 134.4, 133.6, 133.3, 132.0, 124.5, 109.6, 85.3, 48.0, 42.5, 29.8, 29.0, 28.0, 26.2 and 26.0; MS m/z 525.6 ($M^+ + 1$), 425.2 (100%), 327.2, 285.2, 186.0.

*Synthesis of N-{6-[imidazo[1,2-d][1,2,4]thiadiazol-3-yl(methyl)amino]hexyl}-2-nitrobenzenesulfonamide (**14**)*:

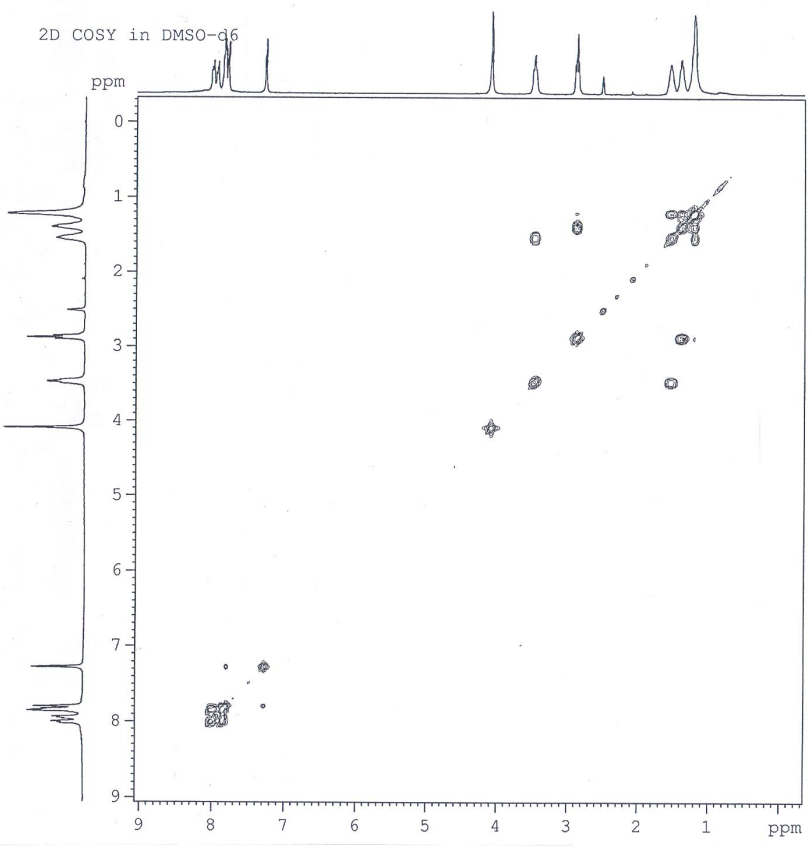
tert-Butyl 6-[imidazo[1,2-*d*][1,2,4]thiadiazol-3-yl(methyl)amino]hexyl[(2-nitrophenyl) sulfonyl]carbamate (300 mg, 0.56 mmol) was dissolved in MeOH (25 mL) and then cooled in ice. HCl gas was bubbled through the solution for ca. 6 mins and the resulting mixture was allowed to warm to room temperature and stirred for 16 h. Volatile materials were removed in vacuo and the residue was purified by column chromatography on silica gel using a solvent mixture of 5% MeOH in CH_2Cl_2 as eluent, thus affording the title compound **14** (240 mg) as a light yellow oil.Yield: 98.4%; ^{1}H-NMR ($CDCl_3$) δ ppm: 8.13-8.15 (m, 1H), 7.85-7.87 (m, 1H), 7.73-7.75 (m, 2H), 7.50 (br.s, 1H), 7.38 (br.s, 1H), 5.31-5.33 (m, 1H, N*H*), 3.40-3.42 (t, J=7.4 Hz, 2H), 3.16 (s, 3H, NC*H*$_3$), 3.08-3.12 (q, J=6.5 Hz, 2H), 1.62-1.68 (m, 2H), 1.53-1.58 (m, 2H) and 1.34-1.39 (m, 4H); MS m/z 439 ($M^+ + 1$), 341(100%), 186, 139.

*N^2-Imidazo[1,2-d][1,2,4]thiadiazol-3-yl-N^2-(6-{[(2nitrophenyl)sulfonyl]amino}hexyl) glycinamide (**16**):*

^{1}H-NMR ($CDCl_3$) δ ppm: 8.15-8.13 (m, 1H), 7.88-7.86 (m, 1H), 7.76-7.75 (m, 2H), 7.45 (s, 1H), 7.39 (s, 1H), 6.55 (br.s, 1H, CONH), 5.67 (br.s, 1H, CONH), 5.45-5.44 (m, 1H, NH), 4.14 (s, 2H, NCH_2CO), 3.59-3.55 (t, J=7.6 Hz, 2H), 3.13-3.09 (q, J=6.4 Hz, 2H), 1.73-1.72 (m, 2H), 1.60-1.57 (m, 2H) and 1.41-1.40 (m, 4H); MS m/z 482(M^++1), 384(100%), 314.

*N-Imidazo[1,2-d][1,2,4]thiadiazol-3-yl-N-(6-{[(2-nitrophenyl) sulfonyl] amino} hexyl) glycine (**17**):*

^{1}H-NMR (DMSO-d_6) δ ppm: 8.00-8.07 (m, 1H), 7.90-7.97 (m, 1H), 7.77-7.83 (m, 2H), 7.90 (s, 1H), 7.28 (s, 1H), 4.15 (s, 2H, NCH_2CO), 3.42-3.54 (t, J=7.2 Hz, 2H), 2.82-2.92 (t, J=6.6 Hz, 2H), 1.50-1.65 (m, 2H), 1.30-1.45 (m, 2H) and 1.15-1.28 (m, 4H); ^{13}C-NMR (DMSO-d_6) δ ppm: 172.0, 158.8, 147.8, 146.2, 137.0, 133.9, 132.9, 132.5, 129.4, 124.3, 114.1, 52.5, 50.9, 42.6, 29.0, 27.1, 25.7 and 25.6; MS m/z 483 (M^++1), 385, 314(100%), 142, 99. The 2D-COSY, 2D-HSQC and 2D-HMBC spectra (**Figures 1-3**) are attached.

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TD                 1024
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DE               252.86 usec
TE                300.0 K
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GB                    0
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F1 - Processing parameters
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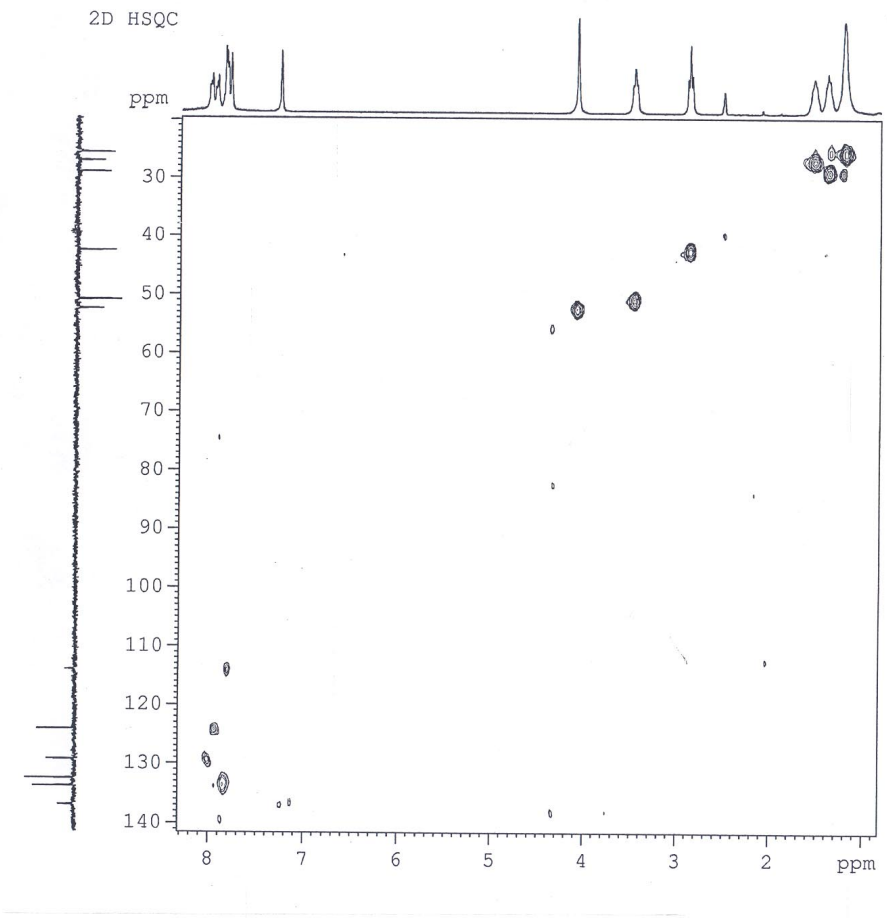
Figure 1. 2D-COSY Spectrum of compound **17**.

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GB                    0
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F1 - Processing parameters
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LB                 0.00 Hz
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2D NMR plot parameters
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F2PLO              8.215 ppm
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F1LO           10428.10 Hz
F1PHI            111.808 ppm
F1HI            8438.06 Hz
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F2HZCM          25.74741 Hz/cm
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Figure 2: 2D-HSQC Spectrum of compound **17**.

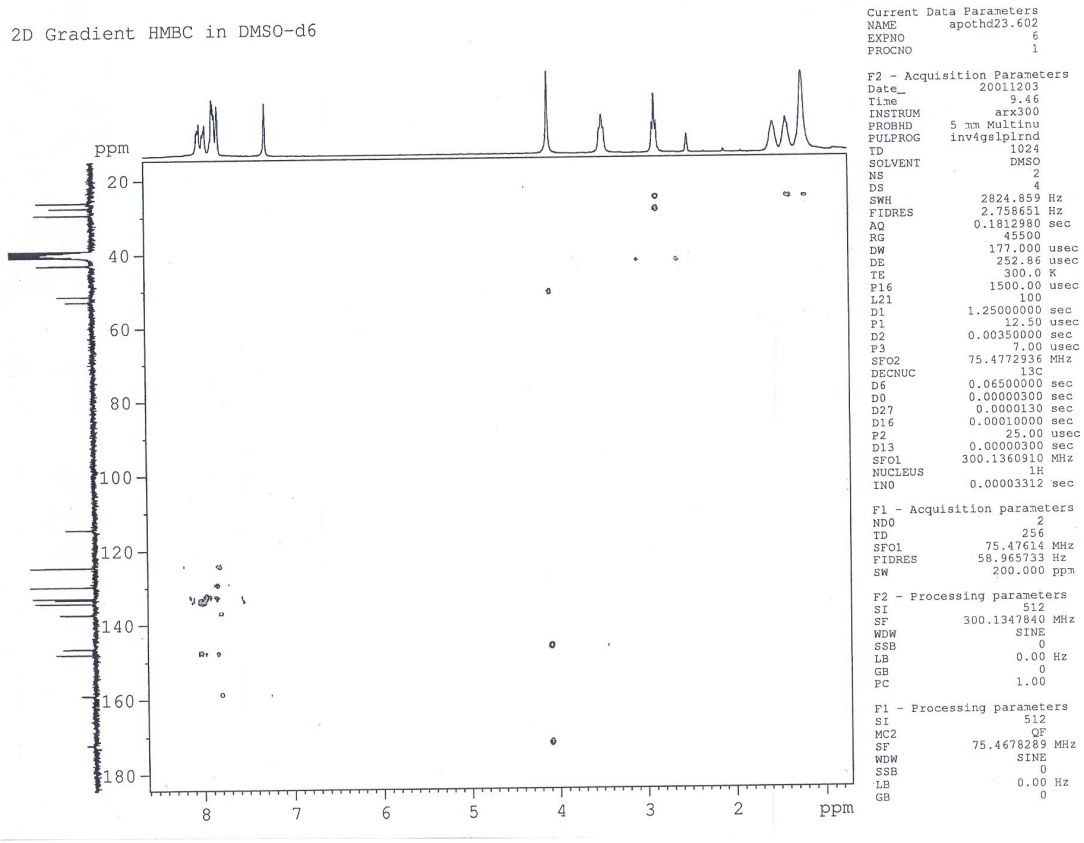

Figure 3: 2D-HMBC Spectrum of compound **17**.

Table 1: HPLC purity, HRMS (ESI), and Combustion Analysis of New Compounds

Compound #	purity (%)[a] (Method 1)[b]	purity (%)[a] (Method 2)[c]	HRMS (ESI)[f]		Elemental Analysis		
			Calc. + H^+	Found + H^+	Calc. for	Calc. C; H; N	Found[g] C, H, N
4	98.8[d]	98.8[e]	240.1283	240.1300	$C_{10}H_{17}N_5S.0.2H_2O$	49.44; 7.22; 28.83	49.84; 7.26; 28.34
5	97.8	99.6	380.1215	380.1223	$C_{16}H_{21}N_5O_2S_2.0.2H_2O$	50.16; 5.63; 18.28	50.48; 5.85; 17.87
6	98.7	99.4	394.1371	394.1372	$C_{17}H_{23}N_5O_2S_2.$ 0.2EtOH	51.89; 6.06; 17.39	52.22; 6.43; 17.57
7	99.4	99.0	394.1371	394.1384	$C_{17}H_{23}N_5O_2S_2.$ 0.3EtOH	51.89; 6.14; 17.19	52.01; 6.20; 17.22
8	98.2	98.5	425.1066	425.1086	$C_{16}H_{20}N_6O_4S_2$	45.27; 4.75; 19.80	45.17; 4.99; 19.54
9	98.9	98.5	425.1066	425.1071	$C_{16}H_{20}N_6O_4S_2$	45.27; 4.75; 19.80	45.46; 5.05; 19.51
10	98.4	99.0	425.1066	425.1053	$C_{16}H_{20}N_6O_4S_2$	45.27; 4.75; 19.80	45.49; 5.06; 19.67
11	98.2	98.1	287.1066	287.1077	$C_{12}H_{18}N_2O_4S$	50.33; 6.34; 9.78	50.81; 6.57; 9.78
12					$C_{17}H_{21}N_5O_6S.$ 0.15hexane	49.27; 5.34; 16.05	49.13; 5.52; 16.05
14	96.3	96.4	439.1222	439.1199	$C_{17}H_{22}N_6O_4S_2$		n/a
17	97.0	96.7	483.1121	483.1129	$C_{18}H_{22}N_6O_6S_2$		n/a

[a] All % reported here are at λ = 254 nm.

[b] Method 1: Waters Symmetry, C18, 3.9 ×150 mm, part #: WAT046980; Temperature = Room temperature; Flow rate = 1 mL/min; Online solvent gradient [ACN/ solution A (0.035% $HClO_4$ solution)] = 0 – 2 minutes: 100% solution A, 2 – 5 minutes: 10/90 ACN/solution A, 5 – 10 minutes: 50/50 ACN/solution A, 10 – 15 minutes: 50/50 ACN/solution A, 15 – 20 minutes: 80/20 ACN/solution A, 20 – 25 minutes: 80/20 ACN/solution A; Monitoring wavelengths (λ): 210 nm, 254 nm, 280 nm, 320 nm.

[c] Method 2: Waters Xterra, C18, 4.6 × 250 mm, Part #: W12881; Temperature: Room temperature; Flow rate: 1 mL/min; Online solvent gradient: [ACN/ solution B (0.1% formic acid solution)] = 0 – 5 minutes: 15/85 ACN/solution B, 5 – 10 minutes: 50/50 ACN/solution B, 10 – 15 minutes: 50/50 ACN/solution B, 15 – 20 minutes: 85/15 ACN/solution B, 20 – 25 minutes: 85/15 ACN/solution B; Monitoring wavelengths (λ): 210 nm, 254 nm, 280 nm, 320 nm.

[d] Method 3: Waters Symmetry, C18, 3.9 ×150 mm, part #: WAT046980; Temperature = Room temperature; Flow rate = 1 mL/min; Online solvent gradient [ACN/ solution A (0.035% $HClO_4$ solution)] = 0 – 2 minutes: 5/95 ACN/solution A, 2 – 7 minutes: 20/80 ACN/solution A, 7 – 10 minutes: 20/80 ACN/solution A; Monitoring wavelengths (λ): 210 nm, 254 nm, 280 nm, 320 nm.
[e] Method 4: Waters Xterra, C18, 4.6 × 250 mm, Part #: W12881; Temperature: Room temperature; Flow rate: 1 mL/min; Online solvent gradient: [ACN/ solution B (0.1% formic acid solution)] = 0 – 2 minutes: 5/95 ACN/solution B, 2 – 7 minutes: 30/70 ACN/solution B, 7 – 12 minutes: 30/70 ACN/solution B; Monitoring wavelengths (λ): 210 nm, 254 nm, 280 nm, 320 nm.

[f] The high resolution MS measurements used leucine enkephalin as a lock mass.

[g] n/a = not available