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Supporting Information

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Sensitized Detection of Inhibitory Fragments and Iterative Development of Non-Peptidic Protease Inhibitors by Dynamic Ligation Screening

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*** Dedicated to Prof. Günther Jung on the occasion of his 70th birthday.

Reagents and General Methods. Fmoc Rink amide resin (0.7 mmol/g), 2-chlorotrityl chloride resin (1.6 mmol/g), Fmoc Sieber Amide resin (0.71 mmol/g) and Fmoc-amino acids were purchased from Novabiochem (Läufelfingen, Switzerland). Anhydrous *N*,*N*-dimethylformamide (DMF), dichloromethane (DCM), tetrahydrofurane (THF), *N*-ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide (EDC), diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBt), *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), acetic anhydride (Ac_2O), acetic acid (AcOH), trifluoroacetic acid (TFA), triisopropylsilane (TIPS), 7-amino-4-methyl-3-coumarinylacetic acid (AMCA), 9-fluorenyl-methoxycarbonyl chloride (Fmoc-Cl), trichlorsilane (Cl_3SiH), acetonitrile (AcCN), triphenylphosphine polystyrene resin were from Sigma Aldrich. 3,4-Diaminofurazan was obtained from Acros Organics. Fmoc solid-phase peptide synthesis was conducted manually employing plastic syringes equipped with PE filters. Bioassaying of SARS-CoV M^{pro} was performed on a microtiter plate reader (SAFIRE, TECAN, Grailsheim, Germany). SARS-CoV M^{pro}, which contained a C-terminal His-tag, was expressed and purified as described.[1] The NMR measurements were conducted on a Bruker Avance 300 MHz spectrometer. For HPLC analysis, an Agilent 1100 system was used with a reversed-phase column (Nucleosil 100 C-18, 5 µm, 2x250 mm, Grom, Herrenberg, operated with acetonitrile-water mixtures containing 0.1 % formic acid), a diode array detector and an ESI-MS employing single quadrupole detection.

Kinetic analysis of fluorogenic substrate. The activity of SARS-CoV M^{pro} was determined by measuring the release of AMCA. The excitation wavelength was set to 380 nm and the emission wavelength to 460 nm; relative fluorescence unit (RFU) λ_{em} 460 nm 63.861 RFU/µM AMCA. The sequence of the fluorogenic substrate used was Ac-TSAVLQ↓AMCA. Cleavage reactions were incubated at 298 K and contained 1 µM SARS-CoV M^{pro}, 100 mM MES pH 7.0, and different concentrations of the fluorogenic substrate (0.25 mM – 2.5 mM) in a total volume of 20 µl. All measurements were carried out on a TECAN SAFIRE (Grailsheim, Germany). The K_M and v_{max} values for the cleavage of fluorogenic substrates by SARS-CoV M^{pro} were determined by measuring and plotting the initial rate, *v*, over a range of substrate concentrations *S* and fitting the data directly into the Lineweaver-Burk-plot.

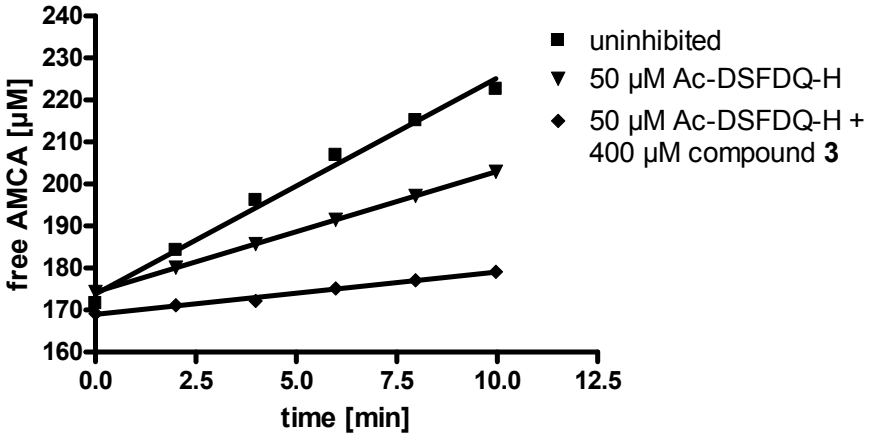


Figure 1: For monitoring the enzymatic reaction, cleavage of the substrate **1** was recorded over time by excitation at 380 nm and emission at 460 nm for released AMCA. The inhibitory effect of fragment **3** was detected as a significant decrease in the rate of the enzyme reaction compared to the negative control (no inhibitor) and to the positive control (peptide aldehyde inhibitor Ac-DSFDQ-H **2** without the addition of a nucleophilic fragment).

Composition of a library of nucleophilic fragments. The subset of nucleophilic fragments used for the screening was derived from the rationally assorted fragment-based screening collection of the Leibniz Institute for Molecular Pharmacology, Berlin. The 20,000 low-molecular-weight compounds of this library were selected due to their diverse representation of reportedly bioactive scaffold elements and in compliance with physicochemical criteria, including the Lipinski rules[2].

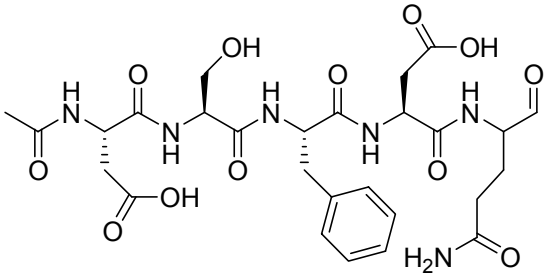
In a first step, approximately 3,000 amines, hydrazines, and thiols among the whole library of 20,000 compounds were identified as nucleophilic fragments. From this rationally composed fragment library containing 3000 nucleophilic fragments, a collection of 234 fragments was selected by a diversity analysis and a subsequent pharmacophoric search using Unity (SYBYL 7.0, Tripos Inc., St. Louis, Missouri, 63144, USA).

Unity is a search and analysis tool for exploring chemical databases. It is capable of finding molecules that satisfy user-defined queries based on molecular fragments, acceptor or donor sites on both molecule or receptor site constraints. The receptors used for the Unity search were the crystal structures 1UK4 (SARS-CoV M^{pro} in complex with the irreversible pentapeptidyl chloromethyl ketone NSTLQ-CH_2-S-Cys145 and 1UJ1 (free enzyme at pH 6.0) published by Yang et al.[3] Finally, as

result of the diversity analysis and pharmacophoric unity search a subset of 234 compounds was selected from the 3,000 nucleophilic fragments.

Bioassay with SARS-CoV M^{pro}. The inhibitory activities found were validated in the established HPLC-based enzyme assay by Tan et al.[4] The sequence of peptide substrate used was SWTSAVLQ↓SGFRKWA-NH_2. Cleavage reactions were incubated at 298 K and contained 1 µM SARS-CoV M^{pro}, 100 mM MES pH 7.0 and 0.5 mM peptide substrate in a total volume of 20 µl. To stop the enzyme reaction, 80 µl of 2% TFA was added and being stored at 193 K. The samples were centrifuged for 10 min at 15,000 *g* before analysis by reverse-phase HPLC on a C18 column (3.9 x 150 mm). Cleavage products were resolved by using a 15 min, 5–60% linear gradient of acetonitrile in 0.1% trifluoroacetic acid. The absorbance was determined at 280 nm, and peak areas were calculated by integration.

Synthesis of fluorogenic substrate Ac-TSVALQ-AMCA (1). The substrate was prepared as described[5] and characterized by HR-ESI-MS. **Ac-TSAVLQ-AMCA:** Yield: 374 mg, 61%. HR-ESI-MS: calcd [M = $C_{40}H_{59}N_9O_{13}$], 873.42323 Da; found (MH^+), *m/z* 874.4160Da.



2

Synthesis of a peptide aldehyde inhibitor (2). Peptide aldehydes were obtained as described in the literature.[6] The product was characterized using HPLC-ESI-MS. **Ac-DSFDQ-H:** Yield: 111 mg, 69%. HR-ESI-MS: calcd [M = $C_{27}H_{36}N_6O_{12}$], 636.23184 Da; found ($M\text{-}H^+$), *m/z* 635.2356, dehydrated product ESI-MS: ($MH^+\text{-}H_2O$), *m/z* 618.0 Da as described in the literature.[5]

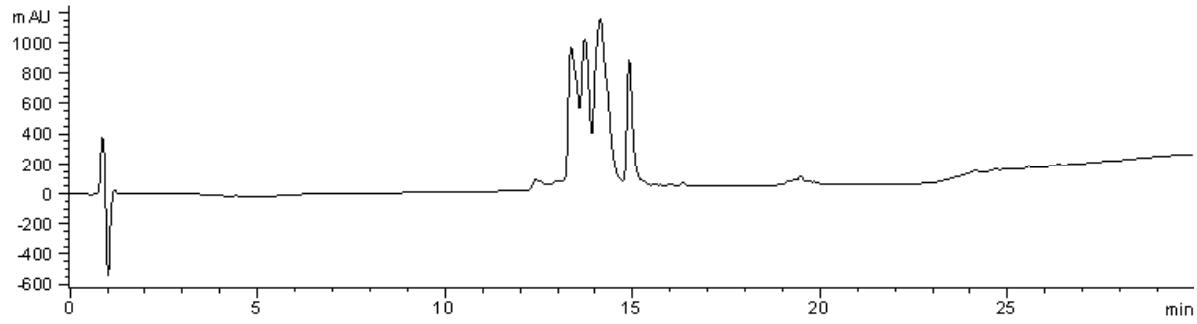
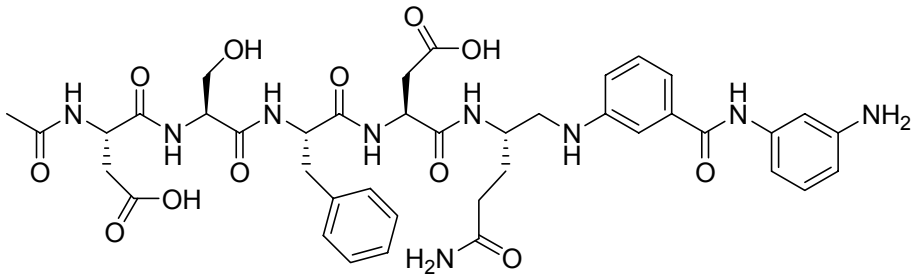


Figure 1 (reproduced from ref. 5). HPLC elution of Ac-DSFDQ-H **2** with an especially "flat" gradient (from 0 % to 25 % acetonitrile in 25 min). Column: Eclipse XDB-C8, Analytical Guard Column, 4,6*12,5 mm, 5 µm (Agilent Technologies); detection at 220 nm; buffer A: 0.1% formic acid in water; buffer B: 0.1% formic acid in ACN. In the first two peaks the MH^+ signal of **2** was observed. The two other peaks displayed the mass of dehydrated peptide aldehyde MH^+-H_2O.

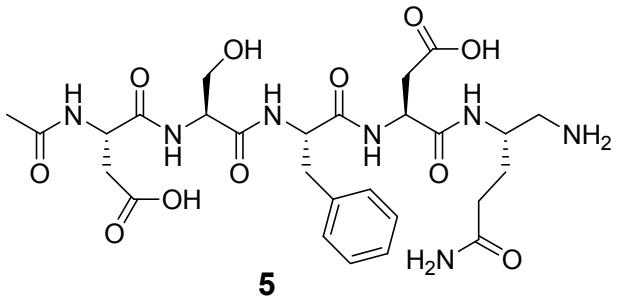


4

Synthesis of *N*-(3-AcDFSDQ(ψCH_2)-aminophenyl)-3-aminobenzamide (4). To a suspension of 1,3-diamino-benzene (1081 mg, 10 mmol) in DCM (2 mL), DIPEA (1.7 mL, 10 mmol) was added and followed by DMF until complete dissolution. The mixture was added to the 2-chlorotrityl resin (1 g) and shaken overnight. Unreacted chlorides were quenched by treatment with methanol for 5 min. Loading was determined from the coupling the Fmoc-Leu by spectrometric Fmoc determination to be 0.2 mmol/g.[7] The free amino group of immobilized *m*-diamino-benzene (1 g, 0.2 mmol/g) was acylated with 3-Fmoc-aminobenzoic acid prepared as described.[5] The 3-Fmoc-aminobenzoic acid (2.88 g, 0.8 mmol), collidine (212 µL, 0.8 mmol), and HATU (304 mg, 0.8 mmol) were solved in DMF and added to the resin, followed by agitation overnight. HPLC-ESI-MS indicated complete conversion.

The Fmoc-(3-amino-*N*-(3-amino-phenyl)-benzamide 2-chlorotrityl resin was Fmoc deprotected by 20% piperidine in DMF for 6 min. Fmoc-Gln(Trt) aldehyde (417 mg,

0.7 mmol) was solved in dry DMF containing 1% acetic acid (AcOH) and was added to the resin (1 g, 0.2 mmol/g). After 10 min the reducing agent $NaCNBH_3$ (132 mg, 2.1 mmol) was added to the reaction vessel. After 3 h the resin was washed. The successful reaction was determined by spectrophotometric Fmoc quantification[7].
Synthesis of the peptide sequence DSFD was carried out manually, according to the DIC/HOBt method as described above. After final Fmoc-deprotection, the N-terminus was acetylated by treatment with Ac_2O (5 eq in 4 mL DMF) for 20 min. Cleavage of the side-chain protecting groups and of the resin was performed in a single step by treatment with a mixture of trifluoroacetic acid/water/triisopropylsilane (95:2.5:2.5, v:v:v) for three hours at room temperature. The crude product was precipitated by adding cold diethylether and collected by centrifugation. The obtained product was lyophilized from water. The product was characterized using HPLC-ESI-MS.
Yield: 95 mg, 55%. ESI-MS: calcd [M = $C_{40}H_{47}N_9O_{13}$], 847.3501 Da; found (MH^+), *m/z* 848.3607 Da.

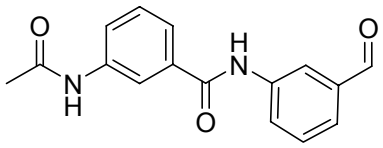


5

Synthesis of Ac-DSFDQ-(ψCH_2-NH_2) (5). The Sieber Amide resin[8] (100 mg, 0.71 mmol/g) was Fmoc-deprotected by 20% piperidine in DMF. Fmoc-Gln-(Trt) aldehyde (417 mg, 0.7 mmol) was solved in dry DMF containing 1% acetic acid (AcOH) and was added to the resin. After 10 min the reducing agent $NaCNBH_3$ (132 mg, 2.1 mmol) was added to the reaction vessel. After 3 h the resin was washed. The successful reaction was determined by spectrophotometric Fmoc quantification.
Synthesis of the peptide sequence DSFD was carried out manually, according to the DIC/HOBt method as described above. After final Fmoc-deprotection, the N-terminus was acetylated by treatment with Ac_2O (5 eq in 4 mL DMF) for 20 min. Cleavage of the side-chain protecting groups and of the resin was performed in a single step by treatment with a mixture of trifluoroacetic acid/water/triisopropylsilane (95:2.5:2.5, v:v:v) for three hours at room temperature. The crude product was precipitated by

adding cold diethylether and collected by centrifugation. The obtained product was lyophilized from water. The product was characterized using HPLC-ESI-MS. **Ac-DSFDQ-(ψCH_2-NH_2):** Yield: 37 mg, 79%. ESI-MS: calcd [M = $C_{27}H_{39}N_7O_{11}$], 637.2701 Da; found (MH^+), *m/z* 638.2701 Da.

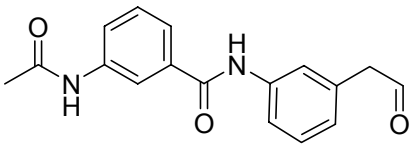
Synthesis of peptides DSFDQ-OH, Ac-DSFDQ-OH, and AcDSFDQ-NH_2. Synthesis of the peptide sequence DSFD was carried out manually, according to the DIC/HOBt method described above, on Rink Amide resin, and 2-chlorotrityl chloride resin. **Ac-DSFDQ-OH**: HR-ESI-MS: calcd [M = $C_{27}H_{36}N_6O_{13}$], 652.2267 Da; found ($M-H^+$), *m/z* 651.2248 Da. **DSFDQ-OH**: HR-ESI-MS: calcd [M = $C_{25}H_{34}N_6O_{12}$], 610.23075 Da; found (MH^+), *m/z* 611.2323 Da. **Ac-DSFDQ-NH_2 (14)**: HR-ESI-MS: calcd [M = $C_{27}H_{37}N_7O_{12}$], 651.25002 Da; found (MH^+), *m/z* 652.2582 Da.



6

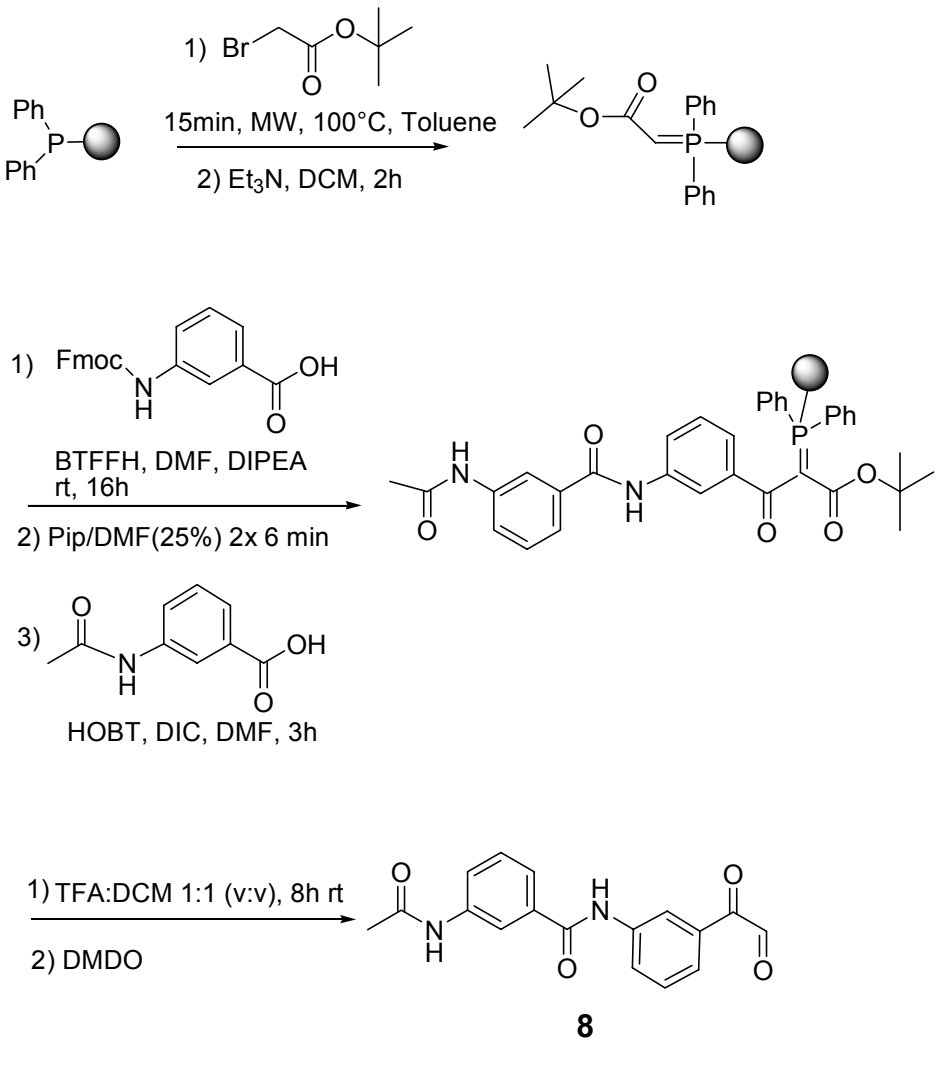
Synthesis of 3-(acetylamino)-*N*-(3-formylphenyl)-benzamide (6). 3-Acetylamino-benzoic acid (197 mg, 1.1 mmol) and EDC (170 mg, 1.1 mmol) were suspended in DCM (6 mL) and stirred for 10 min. Then 3-aminobenzylalcohol (135 mg, 1.1 mmol) was added and stirred overnight. AcOEt (30 mL) was added and the organic phase was washed with 0.1 N HCl (3 x 25 mL) and with saturated Na_2CO_3 solution (3 x 25 mL), dried over $MgSO_4$ and evaporated to yield 3-acetylamino-*N*-[3-(2-hydroxyethyl)phenyl]-benzamide (0.17 mmol, 48 mg). The product was dissolved in DCM (5 mL), Dess-Martin-periodinane (5 eq., 0.9 mmol, 382 mg) was added, followed by 500 µL water. The mixture was stirred until oxidation was completed as indicated by TLC. The work-up was performed by adding 30 mL AcOEt. The organic phase was washed twice with saturated $NaHCO_3$ solution, H_2O, and brine, dried with $MgSO_4$, filtered and evaporated to dryness yielding product **16** (24.8 mg, 52%). ESI-MS: calcd [M = $C_{16}H_{14}N_2O_3$], 282.1 Da; found (MH^+), *m/z* 283.0 Da. ^{1}H-NMR: (300 MHz): δ = 2.85 (s, 3 H, CH_3), 7.42- 7.48 (dd, 1 H, Ar C*H*, *J* = 8.0 Hz, *J'*= 8.0 Hz), 7.56- 7.67 (m, 3 H, Ar C*H*), 7.80- 7.83 (d, 1 H, Ar C*H*, *J* = 8.1 Hz), 8.03- 8.06 (d, 1 H, Ar C*H*, *J* = 8.1 Hz), 8.10 (s, 1 H, Ar C*H*), 8.36 (s, 1 H, Ar C*H*), 10.00 (s, 1 H, NH), 10.13 (s, 1 H, NH), 10.50 (s, 1 H, CH), ppm. ^{13}C-NMR: (300 MHz): δ = 23.95 (CH_3),

118.51, 120.08, 121.94, 122.14, 125.34, 126.02, 128.73, 129.53, 135.24, 136.66, 139.49, 139.94, 165.83 (*C*ONH$_2$), 168.52 (*C*ONH$_2$), 193.01 (*C*HO).



7

Synthesis of 3-(acetylamino)-*N*-[3-(2-oxoethyl)-phenyl]-benzamide (7). 3-Acetylamino-benzoic acid (197 mg, 1.1 mmol) and EDC (170 mg, 1.1 mmol) were suspended in DCM (6 mL) and stirred for 10 min. Then, 2-(3-aminophenyl)-ethanol (151 mg, 1.1 mmol) was added and stirred overnight. The solution was diluted with AcOEt (30 mL) and washed with 0.1 N HCl (3 x 25 mL). Afterwards, the organic phase was washed with saturated Na_2CO_3 solution (3 x 25 mL) and dried over $MgSO_4$. 3-Acetylamino-N-[3-(2-hydroxyethyl)phenyl]-benzamide (0.16 mmol, 48 mg) was obtained by evaporation and dissolved in 5 mL DCM. Dess-Martin-periodinane (5 eq., 0.8 mmol, 339 mg) was added, followed by 500 µL of water. The mixture was stirred until oxidation was complete, as checked by TLC. The work-up was performed by adding 30 mL AcOEt. The organic phase was washed twice with saturated $NaHCO_3$ solution, H_2O, and brine, dried with $MgSO_4$, filtered and evaporated to dryness. Finally, a yield of 8.9 mg, 19% of the desired product was obtained. ESI-MS: calcd [M = $C_{17}H_{16}N_2O_3$], 296.1 Da; found (MH^+), *m/z* 297.0 Da. ^{1}H-NMR: (300 MHz): δ = 2.07 (s, C*H*$_3$), 3.74 (s, C*H*$_2$), 6.96-6.99 (d, Ar C*H*, *J*= 7.2 Hz), 7.29- 7.32 (dd, Ar C*H*, *J*= 2.7 Hz, *J'*= 2.7 Hz), 7.40- 7.45 (dd, Ar C*H*, *J*= 7.8 Hz, *J'*= 7.8 Hz), 7.58- 7.61 (d, Ar C*H*, *J*= 7.5 Hz), 7.65-7.67 (m, Ar *C*H), 7.79-7.81 (d, Ar C*H*, *J*= 7.5 Hz), 8.05 (s, Ar C*H*), 9.69, 10.11, 10.16, 10.23 ppm. ^{13}C-NMR: (300 MHz): δ = 23.95 (*C*H$_3$), 49.66 (CH$_2$), 118.48, 118.91, 121.43, 121.87, 125.02, 128.64, 128.94, 133.10, 135.61, 139.41, 165.55 (*C*ONH$_2$), 168.48 (*C*ONH$_2$), 200.27 (*C*HO) ppm.

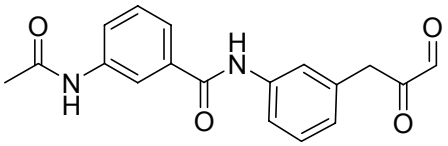


8

Synthesis of 3-(acetylamino)-*N*-[3-(2-oxoacetyl)-phenyl]-benzamide (8). Polymeric *tert*.-butyl phosphoranylidene acetate (700 mg) prepared analogously to the literature[9] was acylated with *N*-Fmoc-3-aminobenzoic acid (2.01 g, 5.6 mmol), BTFFH (1.77g, 5.6 mmol) and DIPEA (1.95 mL, 11.2 mmol) in 8.5 mL of dry DMF for 20h. Then, the resin was washed with DMF and DCM and dried *in vacuo*. The loading was determined by Fmoc cleavage[7] of small aliquots to be 1.37 mmol/g. Following Fmoc group cleavage the resulting free amino group was acylated shaking the resin with 3-acetylaminobenzoic acid (1 g, 5.6 mmol), BTFFH (1.77 g, 5.6 mmol), and DIPEA (1.95 mL, 11.2 mmol) in 6 mL of dry DMF for 6h. After washing with DMF and DCM the resin was treated with 6 mL of TFA:DCM (95:5) for 18 h to remove the *tert*-butyl group. After washing the resin with DCM (5x), neutralization was carried out by treating the resin with DIPEA:DCM (1:9, v:v) (3 x 5 min) followed by more DCM washings. Cleavage of the compound from the resin was carried out by treating 350 mg of the resin solvated in the minimum dry DCM with 5 eq of DMDO (507.5 mg, 6.6

mmol, the volume was calculated based on the concentration of the DMDO[9]) for 1h at 0ºC. The resin was filtered and the filtrate was evaporated to dryness, suspended in H_2O:AcCN (1:1, v:v), frozen and lyophilized. Crude product **8** was obtained (80.2 mg, 80 % purity) as a yellow solid. The product was purified by semipreparative HPLC and characterized by LC-MS and NMR.

ESI-MS: calcd [M = $C_{17}H_{14}N_2O_6$], 310.31 Da; found (MH^+), *m/z* 311.0 Da. ^{1}H-NMR: (300 MHz): δ = 2.20 (s, 3 H, *C*H_3), 7.42- 7.48 (dd, 1 H, Ar C*H*, *J* = 8.0 Hz, *J'*= 8.0 Hz), 7.56- 7.67 (m, 3 H, Ar C*H*), 7.80- 7.83 (d, 1 H, Ar C*H*, *J* = 8.1 Hz), 8.03- 8.06 (d, 1 H, Ar C*H*, *J* = 8.1 Hz), 8.10 (s, 1 H, Ar C*H*), 8.36 (s, 1 H, Ar C*H*), 9.04 (s, 2 H, NH), 9.64 (s, 1 H, CH), ppm. ^{13}C-NMR: (300 MHz): δ = 23.49 O*C*H_3), 117.08, 120.35, 120.38, 124.36, 125.40, 125.44, 128.41, 130.16, 133.81, 134.71, 137.68, 138.85, 164.10 (*C*O), 168.29 (NH*C*O), 189.89 (*C*HO), 190.86 (CHO*C*O) ppm.

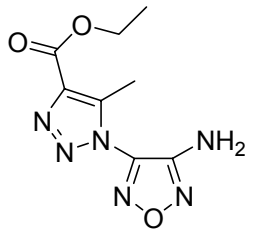


9

Synthesis of 3-(acetylamino)-*N*-[3-(2,3-dioxopropyl)-phenyl]-benzamide (9). Polymeric *tert.*-butyl phosphoranylidene acetate (700 mg) prepared analogously to the literature[9] was acylated with *N*-Fmoc-3-aminophenylacetic acid (2.27 g, 5.6 mmol), BTFFH (1.77g, 5.6 mmol), and DIPEA (1.95 mL, 11.2 mmol) in 8.5 mL of dry DMF for 20h. Then, the resin was washed with DMF and DCM and dried *in vacuo*. The loading was determined by Fmoc cleavage[7] of small aliquots to be 1.37 mmol/g. Following Fmoc group cleavage the resulting free amino group was acylated shaking the resin with 3-acetylaminobenzoic acid (1 g, 5.6 mmol), BTFFH (1.77 g, 5.6 mmol), and DIPEA (1.95 mL, 11.2 mmol) in 6 mL of dry DMF for 6h. After washing with DMF and DCM the resin was treated with 6 mL of TFA:DCM (95:5) for 18 h to remove the *tert*-butyl group. After washing the resin with DCM (5x), neutralization was carried out by treating the resin with DIPEA:DCM (1:9, v:v) (3 x 5 min) followed by more DCM washings. Cleavage of the compound from the resin was carried out by treating 350 mg of the resin solvated in the minimum dry DCM with 5 eq of DMDO (507.5 mg, 6.6 mmol, the volume was calculated based on the concentration of the DMDO[9]) for 1h at 0ºC. The resin was filtered and the filtrate was evaporated to dryness, suspended in H_2O:AcCN (1:1, v:v), frozen and lyophilized. 96 mg (75 %

purity) of the target product as a yellow solid were obtained. The product was purified by semipreparative HPLC and characterized by LC-MS and NMR.

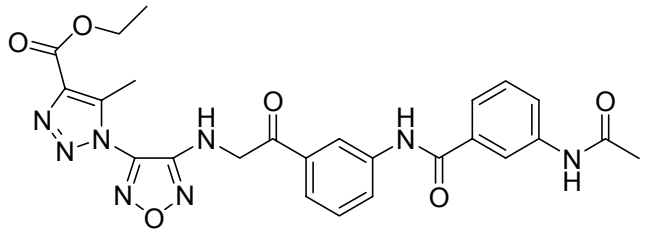
ESI-MS: calcd [M = $C_{18}H_{16}N_2O_6$], 324.34 Da; found (MH^+), *m/z* 325.0 Da. ^{1}H-NMR: (300 MHz): δ = 2.17 (s, C*H*$_3$), 3.74 (s, C*H*$_2$), 6.96-6.99 (d, Ar C*H*, *J*= 7.2 Hz), 7.29-7.32 (dd, Ar C*H*, *J*= 2.7 Hz, *J'*= 2.7 Hz), 7.40- 7.45 (dd, Ar C*H*, *J*= 7.8 Hz, *J'*= 7.8 Hz), 7.58- 7.61 (d, Ar C*H*, *J*= 7.5 Hz), 7.65-7.67 (m, Ar *C*H), 7.79-7.81 (d, Ar C*H*, *J*= 7.5 Hz), 8.05 (s, Ar C*H*), 9.69, 10.11, 10.16, 10.23 ppm. ^{13}C-NMR: (300 MHz): δ = 23.49 O*C*H$_3$), 48.61 (*C*H$_2$), 117.08, 120.35, 120.38, 124.36, 125.40, 125.44, 128.41, 130.16, 133.81, 134.71, 137.68, 138.85, 164.10 (*C*O), 168.29 (NH*C*O), 189.89 (*C*HO), 190.86 (CHO*C*O) ppm.



11

Synthesis of ethyl 1-(4-amino-furazan-3-yl)-5-methyl-1H-[1,2,3]triazole-4-carboxylate (11). The synthesis was performed accorded to a published procedure.[10, 11] 3-Amino-4-azidofurazan was obtained in a yield of 212 mg, 63 %. Observed melting point: 86 °C (literature[10]: mp: 86.5-87.5 °C).

1-(4-Amino-furazan-3-yl)-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid ethyl ester was obtained in a yield of 60 mg, 75 %. Observed melting point: 138 °C (literature[11]: mp: 138-139 °C).



12

Synthesis of ethyl 1-(2-(3-(3-acetamido-benzamido)phenyl)-2-oxo-ethyl-4-amino-furazan-3-yl)-5-methyl-1H-[1,2,3]triazole-4-carboxylate (12). As described in the literature[12] ketoaldehyde **8** (13 mg, 38.9 µmol) and 1-(4-amino-furazan-3-yl)-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid ethyl ester **11** (9.3 mg, 38.9 µmol) were

stirred in dry DMF/MeOH (1:1, v:v) containing 1% AcOH and molecular sieves for 3 h. Then Cl_3SiH (5 eq.) was added and stirred for 1 h. The molecular sieves were filtered off and the filtrate was evaporated in vacuo to dryness. The crude product was purified by preparative HPLC. Finally a yield of 6 mg (29 %) of **12** was obtained. ESI-MS: calcd [M = $C_{25}H_{24}N_8O_6$], 532.53 Da; found (MH^+), *m/z* 533.0 Da. ^{1}H-NMR: (300 MHz): δ = 1.48 (t, CH2C*H*$_3$ *J* = 5.8 Hz), 2.20 (s, COCH3), 2.72 (s, *CH*$_3$), 3.74 (s, CH2), 4.56 (q, C*H*$_2$, *J*= 7.1 Hz), 6.96-6.99 (d, Ar C*H*, *J*= 7.2 Hz), 7.29- 7.32 (dd, Ar C*H*, *J*= 2.7 Hz, *J'*= 2.7 Hz), 7.40- 7.45 (dd, Ar C*H*, *J*= 7.8 Hz, *J'*= 7.8 Hz), 7.46 (s, 3H, NH), 7.58- 7.61 (d, Ar C*H*, *J*= 7.5 Hz), 7.65-7.67 (m, Ar *C*H), 7.79-7.81 (d, Ar C*H*, *J*= 7.5 Hz), 8.05 (s, Ar C*H*) ppm. ^{13}C-NMR: (300 MHz): δ = 7.99 (Ar*C*H$_3$), 13.87 (*C*H$_3$), 23.49 (CO*C*H$_3$), 60.72 (O*C*H$_2$), 117.10, 120.98, 124.36, 125.40, 127.12, 129.48, 130.16, 134.67, 134.71, 138.85, 141.53, 144.23, 162.93 (*C*OO), 164.10 (*C*ONH), 168.29 (*C*OCH$_3$), 168.47 (NH*C*OCH$_2$) ppm.

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