Supplementary Material (ESI) for Soft Matter


**Supporting Information**

# Bioconjugates to Specifically Render Inhibitors Water-Soluble

by **Anna K. H. Hirsch, François Diederich, Markus Antonietti and Hans G. Börner**

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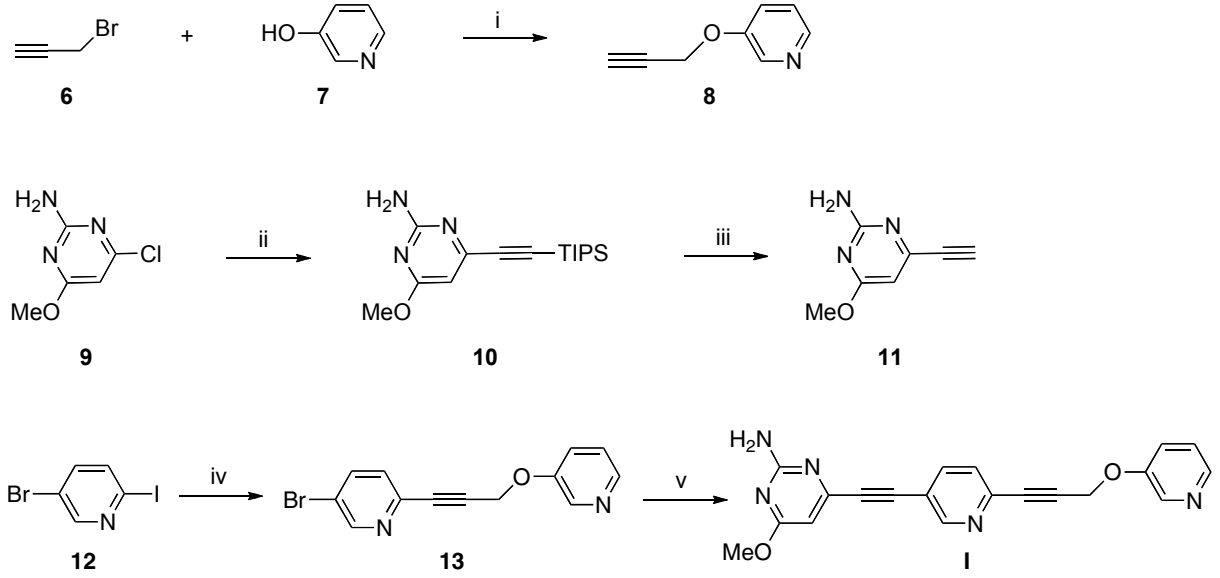
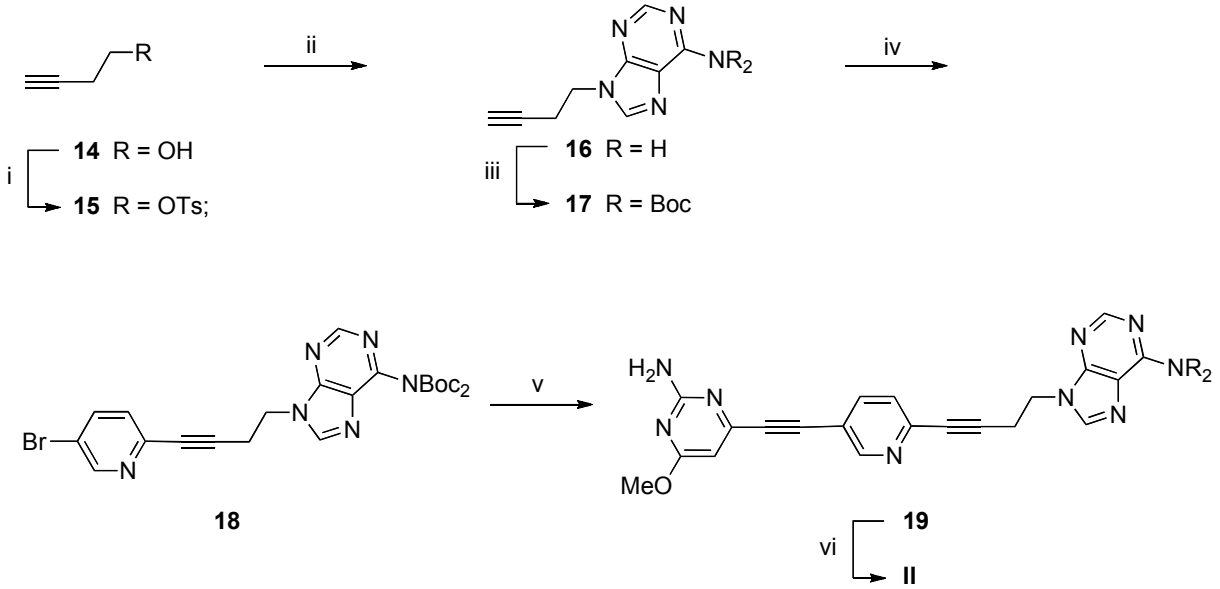
***Table of Contents***




**Scheme 1ESI.** Synthesis of bisubstrate inhibitor **I**. (i) $Cs_2CO_3$, DMF, 0 °C, 1 h, 46%; (ii) TIPS-acetylene, $Et_3N$, $[PdCl_2(PPh_3)_2]$, CuI, DMF, 70 °C, 16 h, 94%; (iii) $^{n}Bu_4NF$, THF, 0 °C, 15 min, quant.; (iv) **8**, $^{i}Pr_2NH$, $[PdCl_2(PPh_3)_2]$, CuI, THF, 25 °C, 22 h, 67%; (v) **11**, $Et_3N$, $[PdCl_2(PPh_3)_2]$, CuI, DMF, 70 °C, 19 h, 37%. DMF = *N,N*-dimethylformamide, TIPS = triisopropylsilyl.




**Scheme 2ESI.** Synthesis of bisubstrate inhibitor **II**. (i) *p*-TsCl, pyridine, $CH_2Cl_2$, 25 °C, 40 h, 41%; (ii) adenine, NaH, DMF, 25 °C, 22 h, 64%; (iii) $Boc_2O$, DMAP, THF, 25 °C, 23 h, quant.; (iv) **12**, $Et_3N$, $[Pd(PPh_3)_4]$, CuI, THF, 25 °C, 18 h, quant.; (v) **11**, $Et_3N$, $[Pd(PPh_3)_4]$, CuI, THF, 70 °C, 19 h, 34%; (vi) TFA, HCl (cat.), $H_2O$, 25 °C, 7 h, 88%. DMAP = 4-dimethylaminopyridine; Ts = *p*-toluenesulfonyl.

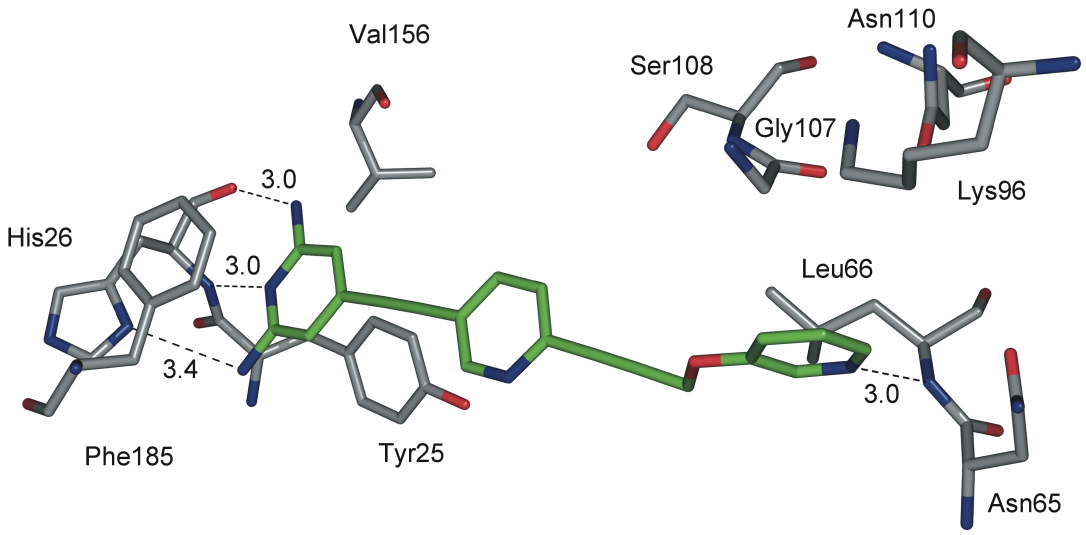



**Fig. 1ESI.** MOLOC-generated molecular model of inhibitor **I** in the active site of *E. coli* IspE (PDB code: 1OJ4).[1] Colour code: protein skeleton: C: grey; inhibitor skeleton: C: green; O: red; N: blue. Hydrogen bonds are indicated as dashed lines. Distances are given in Å.

**Experimental Section**

**Modelling of the Inhibitors Using MOLOC:** Potential inhibitors were manually docked into the active site of IspE.[1] The structure of the ternary complex of *E. coli* IspE (PDB code: 1OJ4) was used. The enzyme coordinates were fixed, and the energy of the system was minimised using the MAB force field as implemented in the computer programme MOLOC.[2] The following factors were used to evaluate the binding mode of the inhibitors: i) avoiding unfavourable repulsive contacts, ii) close examination of the hydrogen bonds and secondary electrostatic interactions, iii) careful conformational analysis of the optimised inhibitors and iii) optimal filling of the binding pockets while maximising hydrophobic contacts between enzyme and ligand.

**Materials for inhibitor synthesis:** Compounds **III**, **IV**, **8** and **15** as well as carrier **5** [DGRDGRDGRDGRDGR-$PEO_{72}$] were prepared according to procedures reported in the literature.[3–5] Solvents and reagents were purchased reagent-grade and used without further purification. All reactions were carried out under an Ar atmosphere, unless otherwise stated. $CH_2Cl_2$ was freshly distilled over $CaH_2$. All products were dried under high vacuum ($10^{-2}$ Torr) before analytical characterisation. TLC: Aluminium sheets coated with $SiO_2$-60 $UV_{254}$ from *Macherey-Nagel*, visualisation by UV light at 245 nm and staining with a solution of $KMnO_4$ (1.5 g), $K_2CO_3$ (10 g), 5% NaOH (2.5 mL) in $H_2O$ (150 mL); or a solution of ninhydrin (0.3 g) in butanol (100 mL) and glacial acetic acid (3 mL). Column chromatography (CC): $SiO_2$-60 (230–400 mesh, 0.040-0.063 mm) from *Fluka*.

**Materials for peptide synthesis**: Diisopropylethylamine (DIPEA; Acros, peptide grade), piperidine (Acros; peptide grade) and trifluoracetic acid (TFA; Acros, peptide grade) were used as received. Dichloromethane (DCM; IRIS Biotech GmbH, peptide grade) was distilled prior to

use. All other reagents were used as received from Aldrich without further purification.

Fmoc-amino acid derivatives (Fmoc-Thr(*t*Bu) OH, Fmoc-Val OH, Fmoc-Gly OH, Fmoc-Phe OH; Fmoc-Ser(*t*Bu) OH; Fmoc-Asn(trt) OH; Fmoc-Asp(*t*Bu) OH; Fmoc-Arg(Pbf) OH), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), and *N*-methyl-2-pyrrolidone (NMP, 99.9+%, peptide synthesis grade) were used as received from IRIS Biotech GmbH, Germany. Fmoc-PNA-G(Bhoc)-OH was used as received from ASM Research Chemicals GmbH & Co. KG (Hannover, Germany). PAP-Resin (poly(ethylene oxide) attached polystyrene-resin; loading: 0.3 mmol·g$^{-1}$; PEO: $M_n = 3200$; $M_w/M_n = 1.04$ (THF GPC) was received from Rapp, Polymere GmbH, Tübingen, Germany.

**Methods:** Melting points (M.p.): *Büchi-510* apparatus; uncorrected. IR Spectra: Perkin Elmer Spectrum BX FTIR System spectrometer (ATR-unit, Attenuated Total Reflection, Golden Gate). NMR spectra ($^1$H, $^{13}$C): Varian Gemini-300, Bruker AMX-400 and Bruker AMX-500; spectra were recorded at 25 °C using the solvent peak as an internal reference. Coupling constants (*J*) are given in Hz. The resonance multiplicity is described as s (singlet), br. s (broad singlet), d (doublet), t (triplet) and m (multiplet). High-resolution mass spectra (HRMS): Varian IonSpec Ultima FT-ICR with 3-hyrdroxypicolinic acid (3-HPA) as matrix (MALDI); Waters Micromass AutoSpec-Ultima (EI). Elemental analyses were performed by the Mikrolabor at the Laboratorium für Organische Chemie, ETH Zürich. The nomenclature was generated with the computer programme ACD/Name (ACD/Labs).

The automated peptide synthesis was performed on an ABI 433a peptide synthesiser (Applied Biosystems, Germany). Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS): Measurements were performed on a Voyager-DE STR

BioSpectrometry Workstation MALDI-TOF mass spectrometer (Perseptive Biosystems, Inc., Framingham, MA, USA). The samples were dissolved in 0.1% TFA in acetonitrile-water (1:1 v/v) at a concentration of 0.1 mg/mL. The analyte solution (1 μL) was mixed with alpha-cyano-4-hydroxycinnamic acid matrix solution (1 μL) consisting of 10 mg of matrix dissolved in 1 mL of 0.3% TFA in acetonitrile-water (1:1 v/v). From the resulting mixture, 1 μL was applied to the sample plate. Samples were air-dried at ambient temperature. Measurements were performed at an acceleration voltage of 20 kV. Each spectrum obtained was the mean of 250 laser shots. Particle-sizer measurements have been performed on a NICOMB Submicron Particle Sizer, Model 370 (Nicomb PSS, Santa Barbara USA) equipped with a 5 mWatt laser, samples where measured as used for bio-assay. Apparent concentrations of the inhibitors were estimated by UV spectroscopy. The bio-assay samples where measured at 300 nm. Assuming a constant extinction coefficient in water/DMSO, calibration was based on inhibitor solutions in methanol (concentration range: $c$[inhibitors] = 0.0125–0.1 mg inhibitor per mL solvent).

**Enzyme assays**

**Materials:** [1,3,4-$^{13}C_3$]-CDP-ME was prepared according to a literature procedure.[6] *E. coli* IspE was also obtained according to the published protocol.[7] NADH and phosphoenolpyruvate potassium salt were purchased from Biomol, ATP and pyruvate kinase/lactate dehydrogenase from Sigma-Aldrich.

**Enzyme-coupled photometric assay for $IC_{50}$ determination:** Assay mixtures were prepared as described with some modifications:[6] 60 μL of a solution containing 100 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 2 mM dithiothreitol, 2.5 mM phosphoenolpyruvate potassium salt, 2 mM ATP, 0.46 mM NADH, 1 U of lactate dehydrogenase, 1 U of pyruvate kinase and IspE protein were added to 60 μL of the solutions containing the inhibitor–polymer complexes (final concentration varied from 8–1000 μM) or the polymer alone. The reaction was started by addition of 60 μL of CDP-ME (final concentration 1 mM) and monitored at 340 nm.

**General Procedure 1 (GP1) for a *Sonogashira* cross coupling:**

To a degassed solution of the aryl halide (1.0 eq.), the acetylene (1.0–3.0 eq.) and the base (2.0–3.0 eq.) in anhydrous DMF or THF, $[PdCl_2(PPh_3)_2]$ or $[Pd(PPh_3)_4]$ (0.1 eq.) and CuI (0.2 eq.) were added. The reaction was left to stir at 25–70 °C in the dark for 16–24 h. The resulting mixture was filtered through a plug of silica gel, washed with $CH_2Cl_2$ and concentrated *in vacuo*.

**Preparation of the inhibitors**

**4-Methoxy-6-{(6-[3-(pyridin-3-yloxy)prop-1-yn-1-yl]pyridin-3-yl}ethynyl)pyrimidin-2-amine (I)**

**GP1**, starting from **13** (46 mg, 0.16 mmol), **11** (24 mg, 0.16 mmol), $Et_3N$ (45 μL, 2.0 mmol), $[PdCl_2(PPh_3)_2]$ (11 mg, 0.016 mmol) and CuI (6.1 mg, 0.032 mmol) in anhydrous THF (3.0 mL). The mixture was left to stir at 70 °C for 16 h. Purification by CC ($SiO_2$; $CH_2Cl_2$/MeOH/$Et_3N$ 100:0:1 → 95:5:1) afforded **I** (21 mg, 37%) as a white solid.

M.p. = 176–177 °C; $\nu_{max}$(thin film): 3302, 3173, 2922, 2848, 1653, 1564, 1472, 1374, 1259, 1117, 1023, 754, 630, 1192, 1150 and 690; $\delta_H$(300 MHz, $(CD_3)_2CO$): 3.89 (s, 3H), 5.20 (s, 2H), 6.19 (br. s, 2H), 6.29 (s, 1H), 7.38 (dd, *J* = 5.3, 8.7, 1H), 7.49–7.53 (m, 1H), 7.58 (dd, *J* = 8.2, 1.1, 1H), 7.98 (dd, *J* = 2.2, 8.2, 1H), 8.24 (d, *J* = 5.3, 1H), 8.42 (d, *J* = 2.7, 1H) and 8.73 (dd, *J* = 1.1, 2.2, 1H); $\delta_C$(75 MHz, $CDCl_3$): 45.9, 56.7, 85.3, 86.4, 86.7, 91.9, 101.6, 118.7, 121.7, 123.9, 126.6, 138.5, 139.2, 141.7, 143.1, 150.0, 152.9, 153.8, 162.8 and 171.0; HR-MALDI-MS: calculated for $C_{20}H_{16}N_5O^+$ ($[M+H]^+$): 358.1299; found: 358.1304.

**9-(4-{5-[(2-Amino-6-methoxypyrimidin-4-yl)ethynyl]pyridin-2-yl}but-3-yn-1-yl)-9*H*-purin-6-amine (II)**

To a solution of **19** (14 mg, 0.023 mmol) in TFA/$H_2O$ (1:1, 0.2 mL) at 0 °C, HCl (conc., 1 drop) was added. The mixture was left to stir at 25 °C for 7 h. The resulting mixture was neutralised with aq. NaOH solution (2.0 N) and extracted with $CHCl_3$ (3x). The combined organic phases were washed with sat. aq. NaCl solution (3x), dried over $Na_2SO_4$, filtered and concentrated *in vacuo* to afford **II** (8.3 mg, 88%) as a white solid.

M.p. = 159–160 °C; $\nu_{max}$(thin film): 3322, 3194, 3059, 2924, 2848, 1644, 1558, 1472, 1436, 1373, 1261, 1183, 1119, 1027, 799, 720, 694 and 631; $\delta_H$(300 MHz, $CDCl_3/CD_3OD$ 6:1): 2.94 (t, $J$ = 6.4, 2H), 3.77 (s, 3H), 4.36 (t, $J$ = 6.4, 2H), 6.16 (s, 1H), 7.20 (dd, $J$ = 0.6, 8.1, 1H), 7.69 (dd, $J$ = 2.2, 8.1, 1H), 7.96 (s, 1H), 8.14 (s, 1H) and 8.52 (dd, $J$ = 0.6, 2.2, 1H,); the signals corresponding to the $NH_2$ groups are not visible; $\delta_C$(75 MHz, $CDCl_3$): 20.8, 42.2, 53.4, 84.7, 86.2, 88.7, 91.2, 109.7, 117.9, 118.7, 126.3, 139.2, 140.7, 141.8, 148.5, 149.1, 150.0, 151.9, 152.0, 152.3 and 170.5; HR-MALDI-MS: calculated for $C_{21}H_{18}N_9O^+$ ($[M+H]^+$): 412.1629; found: 412.1622.

**4-Methoxy-6-[(triisopropylsilyl)ethynyl]pyrimidin-2-amine (10)**

**GP1**, starting from 2-amino-6-chloro-4-methoxypyrimidine (**9**) (670 mg, 4.0 mmol), TIPS-acetylene (1.3 mL, 5.6 mmol), $Et_3N$ (1.1 mL, 8.0 mmol), $PPh_3$ (210 mg, 0.80 mmol), $[PdCl_2(PPh_3)_2]$ (560 mg, 0.80 mmol) and CuI (310 mg, 1.6 mmol) in anhydrous DMF (20 mL). The mixture was left to stir at 70 °C for 16 h. Purification by CC ($SiO_2$; EtOAc/cyclohexane/$Et_3N$ 5:95:1 → 15:85:1) afforded **10** (120 mg, 94%) as an off-white, crystalline solid.

M.p. = 80–83 °C; $\nu_{max}$(neat): 3503, 3489, 3283, 3154, 2939, 2888, 2862, 2724, 2166, 2050, 1653, 1625, 1557, 1487, 1465, 1448, 1414, 1358, 1288, 1255, 1207, 1161, 1124, 1065, 1028, 998, 974, 935, 918, 881, 811, 780, 713, 675 and 660; $\delta_H$(300 MHz, $CDCl_3$): 1.08 (s, 21H), 3.86 (s, 3H), 5.36 (br. s, 2H) and 6.22 (s, 1H); $\delta_C$(75 MHz, $CDCl_3$): 11.3 (3C), 18.7 (6C), 53.6, 93.7, 101.5, 104.3, 150.6, 162.8 and 170.6; HR-MALDI-MS: calculated for $C_{16}H_{28}N_3OSi^+$ ($[M+H]^+$): 306.1996; found: 306.2001.

**4-Ethynyl-6-methoxypyrimidin-2-amine (11)**

To a solution of **10** (78 mg, 0.25 mmol) in moist THF (16 mL) at 0 °C, TBAF (1 M in THF, 0.31 mL, 0.31 mmol) was added. The mixture was left to stir at 0 °C for 15 min. Sat. aq. $NH_4Cl$ solution (50 mL) and $CH_2Cl_2$ (200 mL) were added, and the organic phase was washed with sat. aq. $NH_4Cl$ solution (2 x 100 mL) and sat. aq. NaCl solution (2 x 100 mL), dried over $Mg_2SO_4$, filtered and concentrated *in vacuo*. Purification by CC ($SiO_2$; EtOAc/cyclohexane/$Et_3N$ 40:60:1) afforded **11** (38 mg, quant.) as a brown, crystalline solid.

M.p. > 105.6 °C (decomposition); $\nu_{max}$(neat): 3259, 3134, 3102, 2952, 2859, 2743, 2660, 2282, 2113, 1724, 1630, 1548, 1482, 1461, 1444, 1415, 1346, 1255, 1201, 1161, 1113, 1072, 1022, 976, 913, 818, 788, 738, 683, 670, 666 and 611; $\delta_H$(300 MHz, $CDCl_3$): 3.12 (s, 1H), 3.89 (s, 3H), 5.29 (br. s, 2H) and 6.24 (s, 1H); $\delta_C$(75 MHz, $CDCl_3$): 53.8, 78.5, 81.2, 101.5, 149.6, 162.6 and 170.7; HR-EI-MS: calculated for $C_7H_7N_3O^+$ ($[M]^+$): 149.0589; found: 149.0584.

**5-Bromo-2-[3-(pyridin-3-yloxy)prop-1-yn-1-yl]pyridine (13)**

**GP1**, starting from 5-bromo-2-iodopyridine (**12**) (280 mg, 1.0 mmol), **8** (130 mg, 1.0 mmol), $^iPr_2NH$ (0.26 mL, 2.0 mmol), $[PdCl_2(PPh_3)_2]$ (70 mg, 0.10 mmol) and CuI (38 mg, 0.20 mmol) in anhydrous THF (10 mL). The mixture was left to stir at 25 °C for 22 h. Purification by CC ($SiO_2$; EtOAc/cyclohexane/$Et_3N$ 30:70:1 → 40:60:1) afforded **13** (200 mg, 67%) as an off-white solid (Found: C, 54.35; H, 3.6; N, 9.5. Calc. for $C_{13}H_9BrN_2O$: C, 54.00; H, 3.1; N, 9.7).

M.p. = 105–107 °C; $\nu_{max}$(neat) 3034, 2915, 2491, 2444, 2217, 2103–1724, 1566, 1558, 1538, 1473, 1458, 1424, 1388, 1375, 1346, 1259, 1223, 1184, 1130, 1106, 1088, 1057, 1037, 1003, 990, 913, 892, 838, 794, 734, 701, 644 and 603; $\delta_H$(500 MHz, $CDCl_3$): 4.98 (s, 2H), 7.25 (ddd, $J$ = 0.5, 4.7, 8.4, 1H), 7.31 (dd, $J$ = 0.7, 8.3, 1H), 7.35 (ddd, $J$ = 1.3, 3.0, 8.4, 1H), 7.78 (dd,

*J* = 2.4, 8.3, 1H), 8.28 (dd, *J* = 1.3, 4.7, 1H), 8.43 (dd, *J* = 0.5, 3.0, 1H) and 8.64 (dd, *J* = 0.7, 2.4, 1H); $\delta_C$(125 MHz, $CDCl_3$): 56.6, 84.1, 86.1, 120.9, 121.7, 123.9, 128.3, 138.5, 138.9, 140.5, 143.1, 151.3 and 153.8; HR-EI-MS: calculated for $C_{13}H_9BrN_2O^+$ ($[M]^+$): 287.9898; found: 287.9895.

**9-But-3-yn-1-yl-9*H*-purin-6-amine (16)**

To a suspension of adenine (860 mg, 6.4 mmol) and NaH (260 mg, 6.4 mmol) in anhydrous DMF (30 mL), **15** (1.3 g, 5.8 mmol) in anhydrous DMF (5.0 mL) was slowly added. The mixture was left to stir at 25 °C for 22 h and concentrated *in vacuo*. Purification by CC ($SiO_2$; $CH_2Cl_2$/MeOH/$Et_3N$ 4:96:1) afforded **16** (690 mg, 64%) as a white solid (Found: C, 57.95; H, 4.9; N, 37.2. Calc. for $C_9H_9N_5$: C, 57.7, H 4.9, N 37.4).

M.p. = 138–140 °C; $\nu_{max}$(neat): 3217, 3104, 2114, 1667, 1599, 1574, 1514, 1479, 1455, 1417, 1360, 1328, 1307, 1236, 1154, 1073, 1030, 1013, 954, 908, 867, 798, 770, 667 and 642; $\delta_H$(300 MHz, $CDCl_3$): 2.07 (t, *J* = 2.7, 1H), 2.80 (dt, *J* = 2.7, 6.3, 2H), 4.38 (t, *J* = 6.3, 2H), 8.00 (s, 1H) and 8.34 (s, 1H); the signals corresponding to the $NH_2$ group are not visible; $\delta_C$(100 MHz, $(CD_3)_2SO$): 19.0, 41.6, 73.2, 80.8, 118.6, 140.8, 149.4, 152.3 and 155.9; HR-EI-MS: calculated for $C_9H_9N_5^+$ ($[M]^+$): 187.0858; found: 187.0852. The N7-alkylated amine was obtained as a side product.

**Di-*tert*-butyl (9-but-3-yn-1-yl-9*H*-purin-6-yl)imidodicarbonate (17)**

To a suspension of **16** (190 mg, 1.0 mmol) and DMAP (12 mg, 0.10 mmol) in anhydrous THF (5.0 mL), $Boc_2O$ (0.6 mL, 2.6 mmol) was added. The mixture was left to stir at 25 °C for 23 h.

Purification by CC ($SiO_2$; EtOAc/cyclohexane 7:3) afforded **17** (390 mg, quant.) as a colourless, crystalline solid (Found: C, 59.0; H, 6.6; N, 17.8. Calc. for $C_{19}H_{25}N_5O_4$: C, 58.9, H 6.5, N 18.1). M.p. = 95–96 °C; $\nu_{max}$(thin film): 3267, 2979, 2935, 1785, 1750, 1601, 1577, 1506, 1452, 1409, 1394, 1369, 1336, 1313, 1276, 1249, 1211, 1138, 1105, 1034, 945, 913, 883, 850, 824, 795, 745 and 646; $\delta_H$(400 MHz, $CDCl_3$): 1.45 (s, 18H), 2.03 (t, $J$ = 2.6, 1H), 2.83 (dt, $J$ = 2.6, 6.4, 2H), 4.45 (t, $J$ = 6.4, 2H), 8.19 (s, 1H) and 8.86 (s, 1H); $\delta_C$(100 MHz, $CDCl_3$): 20.0, 27.8 (6C), 42.8, 71.9, 79.6, 83.7 (2C), 128.9, 144.9, 150.4, 150.5 (2C), 152.1 and 153.2; HR-EI-MS: calculated for $C_{14}H_{17}N_5O_2^+$ ([$M$–Boc]$^+$): 287.1377; found: 287.1377.

**Di-*tert*-butyl {9-[4-(5-bromopyridin-2-yl)but-3-yn-1-yl]-9*H*-purin-6-yl}imidodicarbonate (18)**

**GP1**, starting from 5-bromo-2-iodopyridine (**12**) (280 mg, 1.0 mmol), **17** (390 mg, 1.0 mmol), $Et_3N$ (0.42 mL, 3.0 mmol), [$Pd(PPh_3)_4$] (27 mg, 0.10 mmol) and CuI (38 mg, 0.20 mmol) in anhydrous THF (10 mL). The mixture was left to stir at 25 °C for 18 h. Purification by CC ($SiO_2$; EtOAc/cyclohexane 3:2) afforded **18** (540 mg, quant.) as a yellow oil.

$\nu_{max}$(thin film): 3726, 3618, 2979, 1748, 1600, 1582, 1489, 1456, 1369, 1254, 1138, 774 and 668; $\delta_H$(400 MHz, $CDCl_3$): 1.44 (s, 18H), 3.07 (t, $J$ = 6.5, 2H), 4.56 (t, $J$ = 6.5, 2H), 7.15 (dd, $J$ = 0.8, 8.3, 1H), 7.74 (dd, $J$ = 2.4, 8.3, 1H), 8.23 (s, 1H), 8.60 (dd, $J$ = 0.8, 2.4, 1H) and 8.87 (s, 1H); $\delta_C$(100 MHz, $CDCl_3$): 21.0, 27.8 (6C), 42.6, 82.6, 83.8 (2C), 86.5, 120.3 (2C), 127.9, 128.9, 138.9, 141.0, 144.8, 150.5 (2C), 151.2, 152.1 and 153.1; HR-MALDI-MS: calculated for $C_{24}H_{27}BrN_6NaO_4^+$ ([$M$+Na]$^+$): 565.1175; found: 565.1169.

**Di-*tert*-butyl [9-(4-{5-[(2-amino-6-methoxypyrimidin-4-yl)ethynyl]pyridin-2-yl}but-3-yn-1-yl)-9*H*-purin-6-yl]imidodicarbonate (19)**

**GP1**, starting from **18** (160 mg, 0.29 mmol), **11** (48 mg, 0.32 mmol), $Et_3N$ (5.0 mL), $[Pd(PPh_3)_4]$ (34 mg, 0.029 mmol) and CuI (11 mg, 0.059 mmol) in anhydrous THF (3.0 mL). The reaction mixture was left to stir at 70 °C for 22 h. Purification by CC ($SiO_2$; EtOAc/cyclohexane 80:20 → 100:0) afforded **19** (61 mg, 34%) as a brown oil.

$\nu_{max}$(thin film): 2976, 2904, 1791, 1750, 1600, 1568, 1472, 1369, 1337, 1249, 1141, 1105, 851, 778 and 668; $\delta_H$(300 MHz, $CDCl_3$): 1.44 (s, 18H), 3.09 (t, $J$ = 6.5, 2H), 3.90 (s, 3H), 4.56 (t, $J$ = 6.5, 2H), 5.30 (br. s, 2H), 6.32 (s, 1H), 7.24 (d, $J$ = 8.0, 1H), 7.75 (dd, $J$ = 2.2, 8.0, 1H), 8.25 (s, 1H), 8.70 (d, $J$ = 2.2, 1H) and 8.88 (s, 1H); $\delta_C$(75 MHz, $CDCl_3$): 20.8, 27.7 (6C), 42.5, 53.6, 83.0, 83.7, 86.4 (2C), 87.7, 91.5, 101.4, 118.0 (2C), 126.1, 128.8, 139.0, 142.0, 144.8, 149.9, 150.3, 152.0, 152.7 (2C), 153.1, 162.7 and 170.8; HR-MALDI-MS: calculated for $C_{31}H_{34}N_9O_5^+$ ($[M+H]^+$): 612.2683; found: 612.2665.

**Synthesis of the PEO–peptide conjugates**

**Solid-phase-supported synthesis of the carriers 1–5.**

The synthesis was performed according to procedures reported elsewhere.[8] Briefly, the PEO-*block*-polypeptide carrier was synthesised *via* solid phase supported peptide synthesis techniques on an ABI 433a peptide synthesiser using NMP as solvent and standard ABI-Fastmoc protocols (single coupling).[9] The PAP resin based on 1% cross-linked polystyrene (PS) was utilised. This resin exhibits cleavable α-hydroxy-ω-amino functionalised $PEO_{72}$ chains that are tethered *via* a benzyl ether linker: $PS_{resin}$-[linker]-$PEO_{72}$-$NH_2$. After stepwise polypeptide assembly utilising HBTU/NMP/piperidine protocols, liberation of the PEO-*block*-polypeptide conjugate was

achieved by cleavage of the benzyl ether linker (99% TFA, 1% TMSBr, 2–6 h). The cleavage mixture was concentrated and precipitated in diethyl ether. The isolated conjugate was dried, redissolved in water with 2% guanidine hydrochloride and dialysed against Millipore water (molecular weight cut-off: 1000 Da), followed by freeze-drying. The chemical identity of **1**–**5**, was confirmed by MALDI-TOF-MS showing appropriate mass signals that could be assigned with an accuracy of 2 Da.

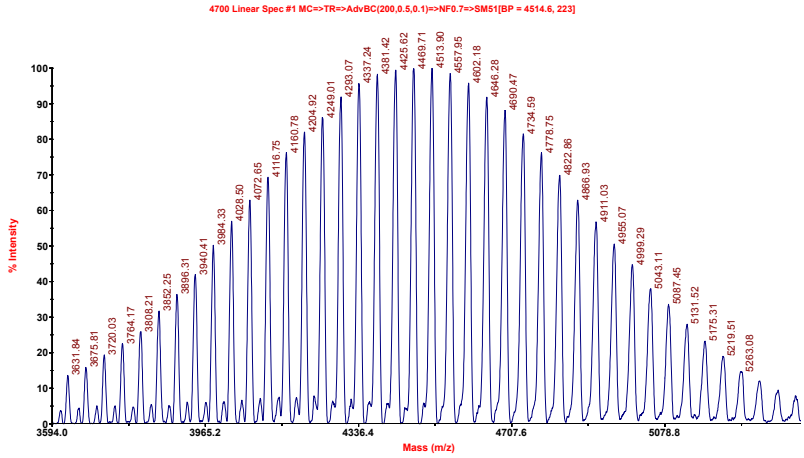
**Characterisation of the PEO–peptide conjugates**

Carrier **1** [RGFNFFSD-$PEO_{72}$]

Average spacing in the m/z distribution could be determined with 44 Da.

$M_{th}$ = 4203.64 Da (with $DP_{PEO}$ = 72); m/z 4205 found correlates to $[M+H]^+$

A minor distribution shifted by 23 Da could be observed that was assignable to $[M+Na]^+$.

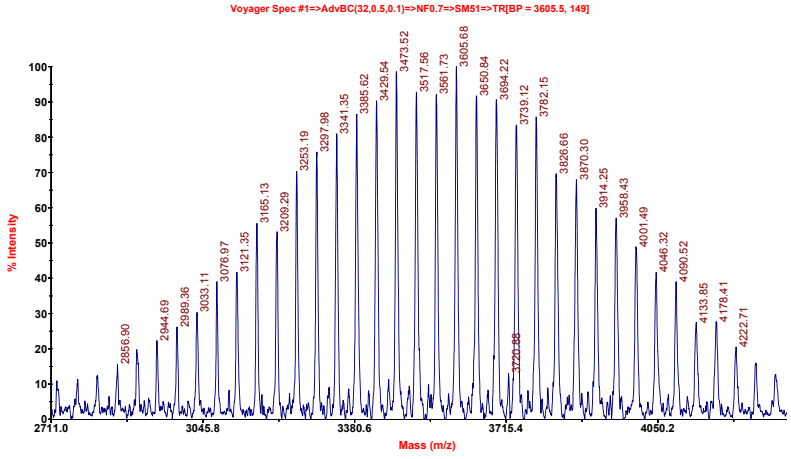



Carrier **2** [PNA*g*-FGFSTN-$PEO_{72}$] (PNA*g*: PNA-guanine residue)

Average spacing in the m/z distribution could be determined with 44 Da.

$M_{th}$ = 3516.27 Da (with *e.g.* $DP_{PEO}$ = 57); m/z 3518 found correlates to $[M+H]^+$

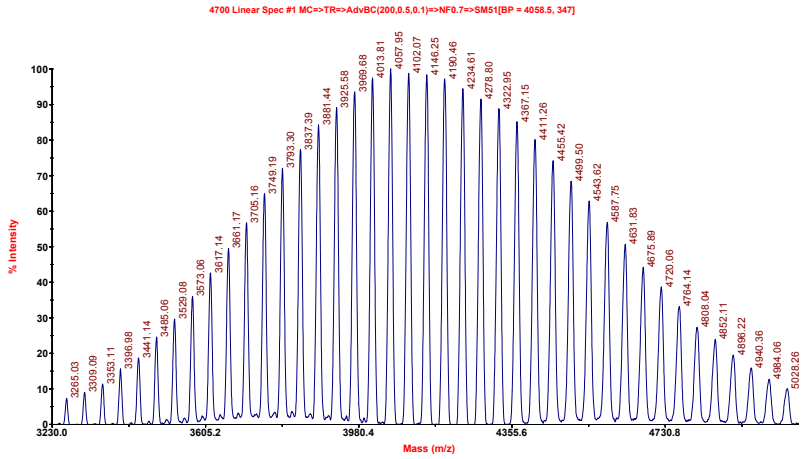
A minor distribution shifted by 23 Da could be observed that was assignable to $[M+Na]^+$.



Carrier **3** [FGRGFNT-$PEO_{72}$]

Average spacing in the m/z distribution could be determined with 44 Da.

$M_{th}$ = 4012.58 Da (with $DP_{PEO}$ = 72); m/z 4013 found correlates to $[M+H]^+$.

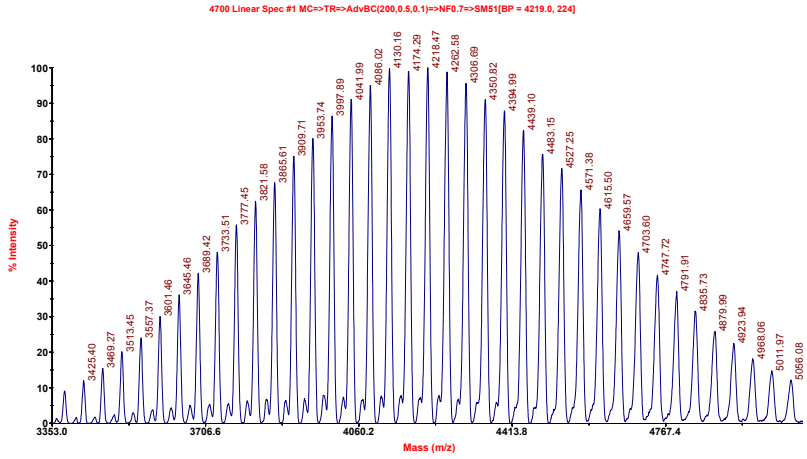



Carrier **4** [Ac-RFGFSTN-$PEO_{72}$]

Average spacing in the m/z distribution could be determined with 44 Da.

$M_{th}$ = 4084.66 Da (*e.g.* with $DP_{PEO}$ = 72); m/z 4086 found correlates to $[M+H]^+$.

A minor distribution shifted by 23 Da could be observed that was assignable to $[M+Na]^+$.



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