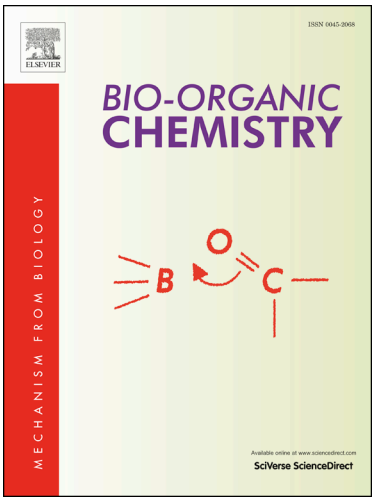


Synthesis and biological evaluation of 7-(aminoalkyl)pyrazolo[1,5-*a*]pyrimidine derivatives as cathepsin K inhibitors

Nejc Petek, Bogdan Štefane, Marko Novinec, Jurij Svete

PII: S0045-2068(18)31124-6
DOI: https://doi.org/10.1016/j.bioorg.2018.11.029
Reference: YBIOO 2637

To appear in: *Bioorganic Chemistry*

Received Date: 3 October 2018
Revised Date: 13 November 2018
Accepted Date: 17 November 2018

Please cite this article as: N. Petek, B. Štefane, M. Novinec, J. Svete, Synthesis and biological evaluation of 7-(aminoalkyl)pyrazolo[1,5-*a*]pyrimidine derivatives as cathepsin K inhibitors, *Bioorganic Chemistry* (2018), doi: https://doi.org/10.1016/j.bioorg.2018.11.029

Synthesis and biological evaluation of 7-(aminoalkyl)pyrazolo[1,5-*a*]pyrimidine derivatives as cathepsin K inhibitors.

Nejc Petek, Bogdan Štefane, Marko Novinec *, and Jurij Svete*

University of Ljubljana, Faculty of Chemistry and Chemical Technology, Večna pot 113, 1000 Ljubljana, Slovenia. E-mail: jurij.svete@fkkt.uni-lj.si

Abstract. A series of novel 7-aminoalkyl substituted pyrazolo[1,5-*a*]pyrimidine derivatives were synthesized and tested for inhibition of cathepsin K. The synthetic methodology comprises cyclization of 5-aminopyrazoles with *N*-Boc-α-*a*mino acid-derived ynones followed by transformation of the ester and the Boc-*a*mino functions. It allows for easy diversification of the pyrazolo[1,5-*a*]pyrimidine scaffold at various positions. Molecular docking studies with pyrazolo[1,5-*a*]pyrimidine derivatives were also performed to elucidate the binding mode in the active site of cathepsin K. The synthesized compounds exhibited moderate inhibition activity ($K_i \geq 77$ μM).

Keywords: pyrazoles; ynones; cyclisation; pyrazolo[1,5-*a*]pyrimidines; cathepsin K; enzyme inhibition; molecular docking.

1. Introduction

Heterocyclic building blocks represent useful scaffolds for applications in medicinal chemistry, catalysis, and materials science [1]. For example, heterocyclic systems that can simulate β-turn structures can serve as replacements of dipeptide motifs in a given native (or natural) peptidic substrate

[2]. An important group of such conformationally constrained U-shaped heterocyclic dipeptide analogues are azabicycloalkane amino acids, which comprise fused heterocycles with a bridgehead nitrogen atom [3].

Among 5–6-fused systems, pyrazolo[1,5-*a*]pyrimidine is an important heterocycle, due to biological activity of many of its derivatives [4]. A SciFinder [5] Substructure search shows around 175,000 known pyrazolo[1,5-*a*]pyrimidine derivatives with over 7,000 references and with preparation, biological study, and uses as the predominant substance roles. For 2017 alone, over 2,000 references can be found for pyrazolo[1,5-*a*]pyrimidines. Examples of bioactive pyrazolo[1,5-*a*]pyrimidines include hepatitis C virus inhibitors [6], antagonists of serotonin 5-HT6 receptors [7], kinase inhibitors [8], PET tumour imaging agents [9], and inhibitors of amyloid β-peptide 1–42 aggregation [10]. Sedative agents zaleplon and indiplon and the anxiolytic agent ocinaplon are approved drugs containing a pyrazolo[1,5-*a*]pyrimidine core (Fig. 1).

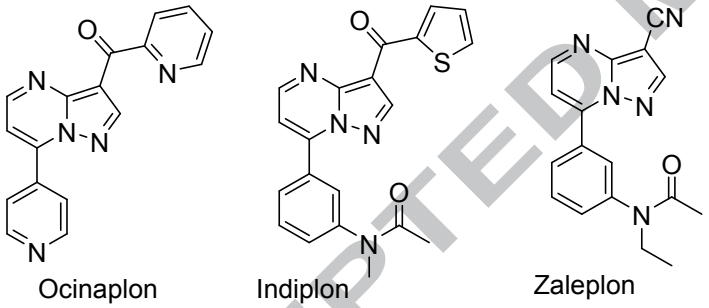


Fig. 1. Approved drugs containing a pyrazolo[1,5-*a*]pyrimidine.

Cathepsin K is a cysteine protease, which is selectively and abundantly expressed within osteoclasts, and which is believed to be crucial for the resorption of bone matrix [11–15]. The ability to degrade type I collagen allows cathepsin K to contribute importantly to the balance between bone resorption and bone formation [16, 17]. It plays an important role in bone resorption and therefore in degenerative bone diseases such as osteoporosis [18].

In contrast to osteoporosis treatments, which mainly utilize estrogen receptor modulators or bisphosphonates accumulation [19], cathepsin K inhibition offers a more controlled treatment. Selective reduction of bone resorption rather than both resorption and formation is possible by inhibition of cathepsin K. Inhibitors of cathepsin K could prevent bone resorption and may provide a promising approach for the treatment of osteoporosis, therefore inhibition of cathepsin K has been proposed as a promising strategy for the treatment of osteoporosis, cancer, and other diseases [11–13]. Several inhibitors have progressed into clinical trials for the treatment of osteporosis as well as other bone and cartilage related diseases but there are, as yet, no inhibitors on the market [19, 20]. The most successful inhibitor was odanacatib (Merck & Co.) USA) [21] which showed robust efficacy in a phase III clinical trial aimed at evaluating osteoporosis-related risk of bone fractures [22]. Unfortunately, it was later terminated due to side effects. Another cathepsin K inhibitor, MIV-711 (Medivir AB, Sweden) [23], is currently in phase II clinical trials for the treatment of osteoarthritis.

Pyrazolo[1,5-*a*]pyrimidines are commonly available by cyclisation of a 3-aminopyrazole derivative with a 1,3-dicarbonyl compound or its synthetic equivalent [24]. Due to this ease of access, a plethora of known pyrazolo[1,5-*a*]pyrimidine derivatives is not surprising. Nevertheless, a more detailed literature search also reveals that only a handful of 7-aminoalkylpyrazolo[1,5-*a*]pyrimidine-3-carboxamides are known [25].

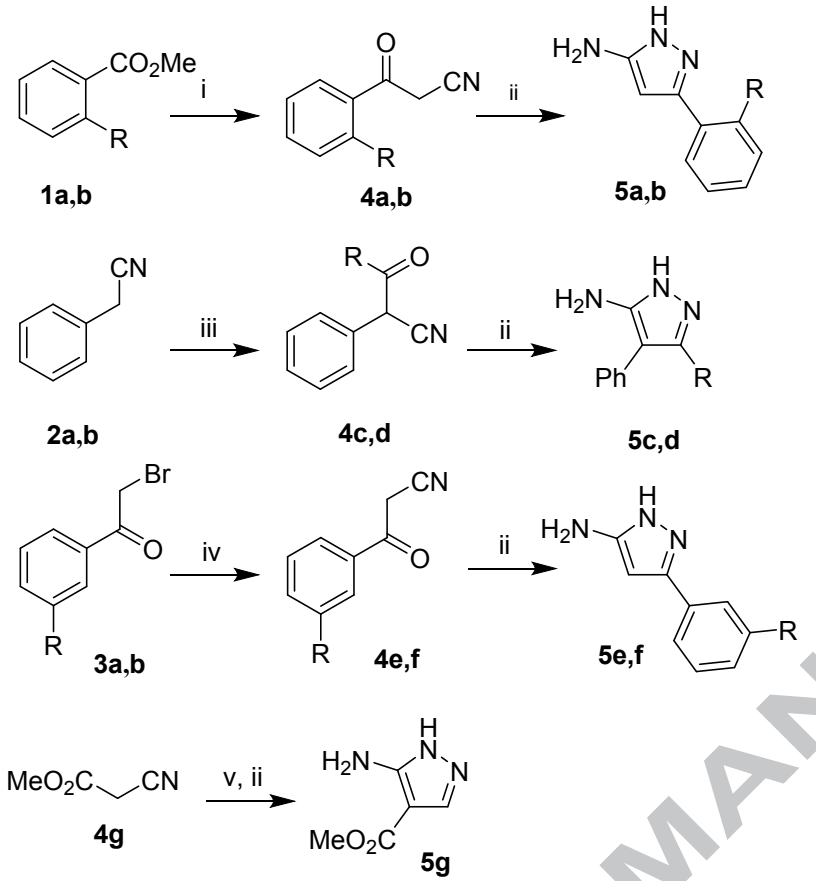
Recently, we focused our attention on the synthesis and transformations of derivatives of pyrazolo[1,5-*a*]pyrimidine-3-carboxamide. Within this context, we reported (parallel) syntheses of libraries of novel 7-heteroarylpyrazolo[1,5-*a*]pyridine-3-carboxamides [26], 7-oxopyrazolo[1,5-*a*]pyrimidine-3-carboxamides [27], 7-(1-aminoethyl)pyrazolo[1,2-*a*]pyrimidines [28], tetrahydropyrazolo[1,5-*c*]pyrimidine-3-carboxamides [29], and 5-(*N*-Boc-*N*-benzyl-2-aminoethyl)-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-3-carboxamides [30]. The latter derivatives also exhibited moderate inhibition of cathepsins B and K [30]. In extension, we elaborated the synthesis of 7-

aminoalkyl substituted pyrazolo[1,5-*a*]pyrimidine derivatives from α-amino acids [28] in terms of scope and structural diversity of the products. A library of pyrazolo[1,5-*a*]pyrimidine derivatives with variable substituents at positions 2, 3, and 7 and with variable degree of saturation of the pyrimidine ring was synthesised and the products were tested for inhibition of cathepsin K. Herein, we report the results of this study.

2. Results and Discussion

2.1. Synthesis of pyrazolo[1,5-a]pyrimidine derivatives

Starting 5-amino-1*H*-pyrazoles **5a**–**g** were prepared by cyclisation of β-ketonitriles **4a–g** with hydrazine hydrate following general literature procedures [31–34]. The β-ketonitriles **4a–f** in turn, were obtained by base-catalysed condensation of alkyl benzoates **1a**,**b** with acetonitrile [35], by acylation of benzyl cyanide **2** [36], and by cyanide displacement of phenacyl bromides **3a**,**b** following literature procedures [37] (Scheme 1, Table 1).



Scheme 1. Synthesis of 5-amino-1*H*-pyrazoles **5a**–**f** from β-keto nitriles **4a**–**f**. Reaction conditions: i) MeCN, *t*-BuOK, toluene, 0 °C; ii) $N_2H_4 \cdot H_2O$, EtOH, reflux; iii) RCO_2Me, EtOH, EtONa, reflux; iv) KCN, EtOH, H_2O, reflux; v) $CH(OEt)_3$, Ac_2O, reflux.

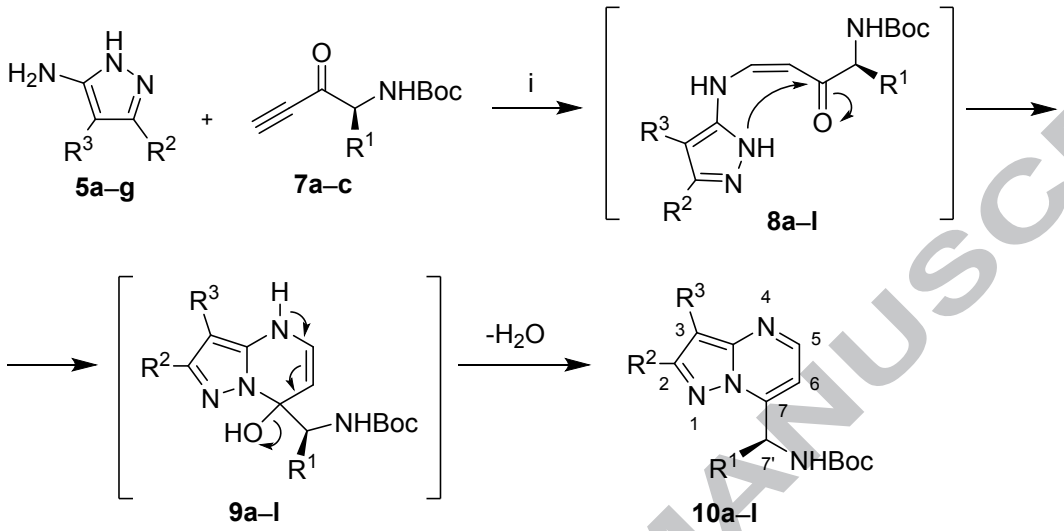
Table 1. Experimental data for β-keto nitriles **4a**–**f** and 5-*a*minopyrazoles **5a**–**f**.

Transformation	R	Yield (%)[a]		Ref.
		4	**5**	
1a→4a→5a	H	46	75 (89)[b]	38
1b→4b→5b	OMe	33	59	39
2a→4c→5c	Me	65	68	40
2b→4d→5d	Bn	96	71 (62)[b]	41
3a→4e→5e	NO_2	70	72	42
3b→4f→5f	CF_3	62	77 (82)[b]	43
4g→5g	-	-	79[c]	44

[a] Isolated yield. [b] Under microwave irradiation. [c] The yield of **5g** obtained in our laboratory; the literature [44] yield is not provided.

The starting ynones **7a–c** were obtained from *N*-Boc-glycine (**6a**), (*S*)-*N*-Boc-alanine, and (*S*)-*N*-Boc-3-phenylalanine (**6c**) following known literature protocol [45]. Also subsequent cyclizations of **7a**–**c** with 5-amino-1*H*-pyrazoles **5a**–**g** and 3-aminoindazole (**5h**) were carried out as described previously for close analogues [28] to afford 7-aminoalkylpyrazolo[1,5-*a*]pyrimidine-3-carboxylates **10a–l** in 20–93% yields. The reaction mechanism can be explained by initial Michael addition of the aminopyrazole **5** to the conjugated triple bond of **7** to give the intermediate enaminone **8** followed by cyclisation to **9** and elimination of water to furnish title compound **10**. Notably, cyclizations of **7a**–**c** with aminopyrazole **5g** in aqueous methanol in the presence of acetic acid (Method B) gave the respective products **10b**,**i**,**l**, in better yields than the reactions in methanol (Method A). This is explainable by the lower nucleophilicity and reactivity of aminopyrazole **5g** in the reactions with 1,3-dielectrophiles **7**, which is due to electron-withdrawing ester group at position 4. Acetic acid activates

the ynone **7** in the Michael addition step and, hence, compensates the lower reactivity of aminopyrazole **5g**. In contrast to our previous observations [28], the cyclizations were regioselective to furnish isomerically pure products **10** isolated upon workup by filtration or by flash chromatography [46] (Scheme 2 and Table 2).



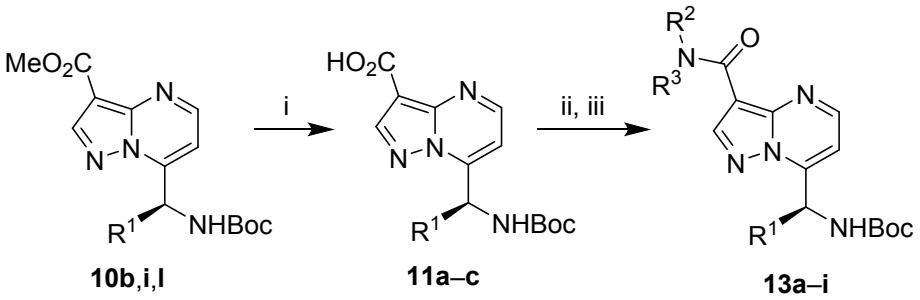
Scheme 2. Cyclizations of ynones **6a**–**c** with 5-aminopyrazoles **5a**–**g**. Reaction conditions: i) MeOH, r.t. (Method A) or MeOH, H_2O, AcOH, r.t. (Method B).

Table 2. Experimental data for 7-(1-aminoalkyl)pyrazole derivatives **10a**–**l**.

Reaction	R^1	R^2	R^3	Yield (%)[a]	Ref.
5a+7a→10a	H	Ph	H	43	[b]
5g+7a→10b	H	H	CO_2Me	74[c]	[b]
5a+7b→10c	Me	Ph	H	93	[b]
5b+7b→10d	Me	2-$MeOC_6H_4$	H	86	[b]
5c+7b→10e	Me	Me	Ph	60	[b]
5d+7b→10f	Me	Bn	Ph	59	[b]
5e+7b→10g	Me	3-$O_2NC_6H_4$	H	57	[b]
5f+7b→10h	Me	3-$F_3CC_6H_4$	H	75	[b]
5g+7b→10i	Me	H	CO_2Me	70[c]	28
5h+7b→10j	Me	–CH=CH–CH=CH–		46	[b]
5a+7c→10k	Bn	Ph	H	48	[b]
5g+7c→10l	Bn	H	CO_2Me	20[c]	[b]

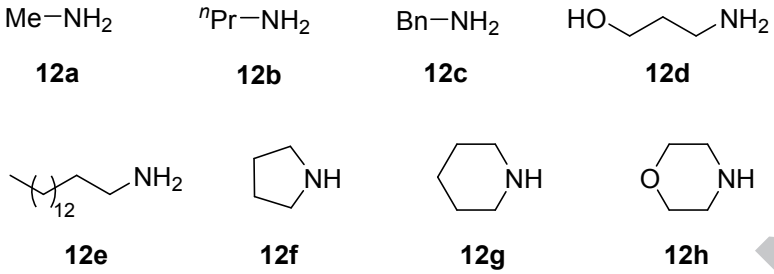
[a] Isolated yield. [b] This paper. [c] Cyclisation was performed in the presence of water and acetic acid.

Base-catalyzed hydrolysis of the pyrazolo[1,5–*a*]pyrimidine-esters **10b**,**i**,**l** afforded the corresponding carboxylic acids **11a**–**c** in 54–89% yields. Next, amidation of **11a**,**b** with primary (**12a**–**e**) and secondary amines **12f**–**h** using 1,1'-carbonyldiimidazole (CDI) as activating reagent furnished carboxamides **13a**–**i** (Scheme 3, Table 3).



10b,**i**,**l** **11a–c** **13a–i**

Amines R^2R^3NH (**12a–h**):



12a **12b** **12c** **12d**

12e **12f** **12g** **12h**

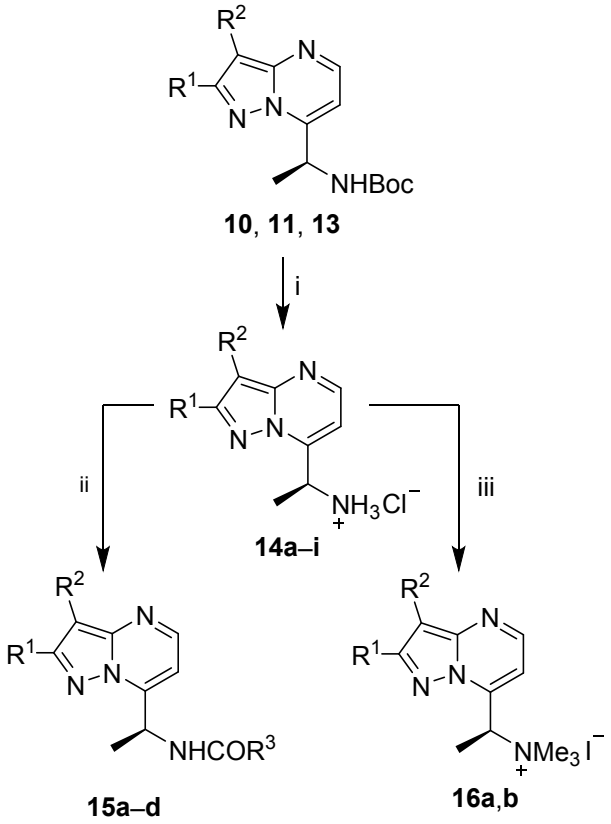
Scheme 3. Synthesis of carboxamides **13a–i**. Reaction conditions: i) $LiOH \cdot H_2O$, MeOH, r.t.; ii) CDI, THF, r.t.; iii) amine **12**, THF, r.t.

Table 3. Experimental data for compounds **11a–c** and **13a–i**.

Reaction	R^1	R^2	R^3	Yield (%)[a]
10b→11a	H	-	-	65
10i→11b	Me	-	-	89
10l→11c	Bn	-	-	54
11a+12a→13a	H	Me	H	49
11b+12a→13b	Me	Me	H	70
11b+12b→13c	Me	*n*-Pr	H	79
11b+12c→13d	Me	Bn	H	100
11b+12d→13e	Me	$(CH_2)_3OH$	H	89
11b+12e→13f	Me	*n*-$C_{15}H_{31}$	H	60
11b+12f→13g	Me	$–(CH_2)_4–$		90
11b+12g→13h	Me	$–(CH_2)_5–$		99
11b+12h→13i	Me	$–(CH_2)_2–O–(CH_2)_2–$		97

[a] Isolated yield.

Next, free amines **14a–i** were prepared from the *N*-Boc analogues **10**, **11**, and **13** by acidolytic deprotection of amino group with HCl–EtOAc. The amines **14** were then N-acylated to give compounds **15a–d** in moderate yields. Quarternization of **14f** and **14j** with excess methyl iodide in the presence of potassium carbonate afforded the quaternary salts **16a** and **16b** in 60% yields (Scheme 4, Table 4).



Scheme 4. Synthesis of amines **14a**–**i**, **16a**,**b** and carboxamides **15a**–**d**. Reaction conditions: i) EtOAc, HCl, r.t.; ii) R_3COCl, Et_3N,CH_2Cl_2, r.t.; iii) MeI, K_2CO_3, r.t.

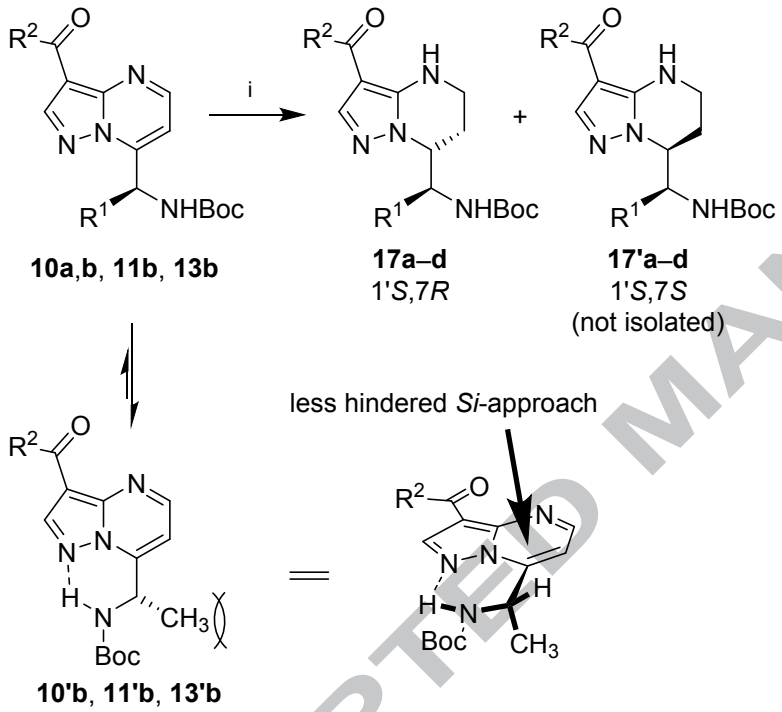
Table 4. Experimental data for compounds **14a**–**i** and **15a**–**d**.

Reaction	R^1	R^2	R^3	Yield (%)[a]
10b→14a	Ph	H		32
10d→14b	2-$MeOC_6H_4$	H		54
10e→14c	Me	Ph		32
10f→14d	Bn	Ph		20
10h→14e	3-$F_3CC_6H_4$	H		100
10i→14f	H	CO_2Me		88
11b→14g	H	CO_2H		51
13b→14h	H	CONHMe		70
13f→14i	H	$CONH(CH_2)_{14}CH_3$		62
14f→15a	H	CO_2Me	Me	48
14f→15b	H	CO_2Me	Ph	85
14f→15c	H	CO_2Me	Bn	68
14h→15d	H	CONHMe	Bn	99
14f→16a	H	CO_2Me		60
14j→16b	H	$CONH(CH_2)_{14}CH_3$		60

[a] Isolated yield.

Somewhat expectedly [28], catalytic hydrogenation of **10a**,**b**, **11b**, and **13b** in the presence of Pd–C under 3 bar of hydrogen afforded the corresponding 4,5;6,7-tetrahydropyrazolo[1,5-*a*]pyrimidines **17a** and **17b**–**d**/**17'b**–**d** in almost quantitative yields. Subsequent chromatographic purification of mixtures of diastereomers **17b–d**/**17'b–d** then furnished pure major (1'*S*,7*R*)-isomers **17b**–**d** in 50–93% yields. Notably, catalytic hydrogenations took place only with the above substrates **10b**,**i**, **11b**, and **13b**

bearing an electron-withdrawing carbonyl group at position 3, whereas attempted hydrogenations of other substrates (**10c**–**h**) were not successful, neither under these reaction conditions, nor in the presence of acetic acid. Diastereoselectivity of hydrogenation is explainable by the less hindered *Si*-attack of hydrogen provided that the substrates **10b**, **11b**, and **13b** adopt a conformation, which is stabilized by an intramolecular hydrogen bond between the amide NH group and the ring nitrogen atom N(1) (Scheme 5, Table 5).



Scheme 5. Synthesis of compounds **17a**–**d**. Reaction conditions: i) H_2 (3 bar), 10% Pd-C, MeOH, r.t.

Table 5. Experimental data for compounds **17a**–**d**.

Compound	R^1	R^2	d.r.[a]	Yield (%)[b]	Ref.
17a	H	OMe	/	96	[c]
17b	Me	OMe	84:16	71	[28]
17c	Me	OH	92:8	93	[c]
17d	Me	NHMe	83:17	50	[c]

[a] Determined from the ^{1}H NMR spectrum of the crude reaction mixture. [b] Isolated yields. [c] This paper.

2.2. Structure determination

The structures of novel compounds **10**, **11**, and **13**–**17** were determined by spectroscopic methods (IR, ^{1}H- and ^{13}C-NMR, and MS-HRMS) and by elemental analyses for C, H, and N. NMR data for compounds **10**, **11**, and **13**–**17** were in agreement with the literature data for related pyrazolo[1,5-*a*]pyrimidines [4, 28]. The structure of compound **17b** was also determined by X-ray diffraction analysis (Figure 2). The detailed structure determination including copies of ^{1}H and ^{13}C NMR spectra are given in the Supporting Information.

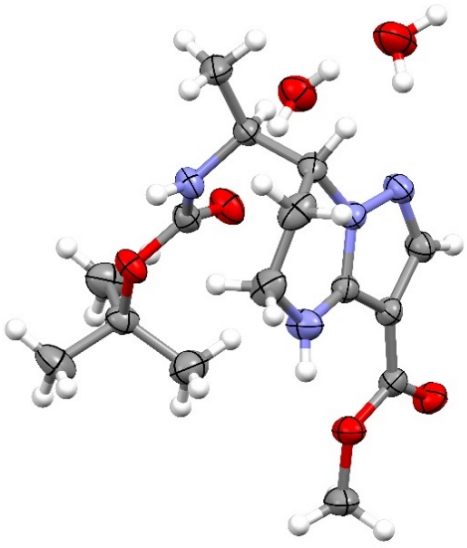


Fig. 2. ORTEP view of molecular structure of compound **17b** and two molecules of cocrystallized water. Displacement ellipsoids are drawn with 40% probability level and the hydrogen atoms are shown as small spheres of arbitrary radii.

2.3. Inhibition of cathepsin K

The synthesized pyrazolo[1,5-*a*]pyrimidines were tested for inhibition of cathepsin K. Due to the absorptive properties of some of the compounds enzymatic activity was followed photometrically using the substrate Z-Phe-arg-*p*NA (benzyloxycarbonyl-Phe-arg-*p*-nitroanilide). Compounds **10a**,**c-h**,**j**,**k** and **13c**,**d** were insoluble in the buffer solution used for kinetic measurements due to their higher lipophilicity. Therefore, we removed the Boc protecting group and synthesized more soluble compounds **14**. A preliminary screen revealed that compounds **10i**, **11b**, **13b**,**e-I** and **15a**,**c** were weak inhibitors of cathepsin K, while compounds **14a-h** showed stronger inhibitory activity. Compound **14f** acted as a stronger inhibitor than its precursor **10i**, indicating additional benefit from removal of the Boc protecting group. Titration curves were recorded for compounds **14a**,**b**,**d**,**e**,**f**,**h** and showed that all compounds acted as linear (full) inhibitors, which is consistent with their binding into the active site of

the protease. Their inhibition constants (K_i) were determined from the titration curves by non-linear regression analysis using the model for linear competitive inhibition (Table 6). The strongest affinity was determined for compound **14d** with a K_i value of 77±5 μM (Table 6, Entry 3).

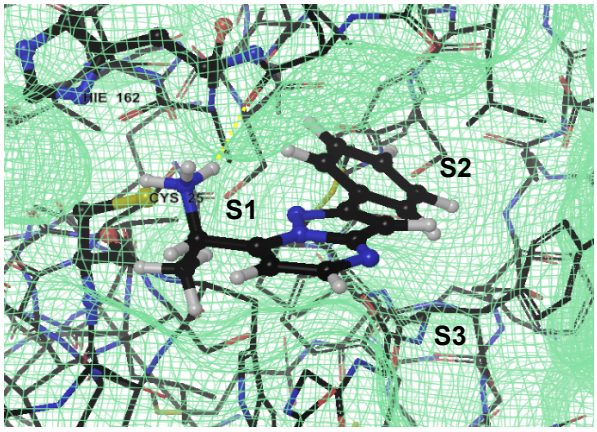
Table 6. Enzyme inhibition data for compounds **14a**,**b**,**d**,**e**,**f**,**h**.

Entry	Compound	R^1	R^2	K_i [μM][a]
1	**14a**	Ph	H	236±15
2	**14b**	2-$MeOC_6H_4$	H	288±27
3	**14d**	Bn	Ph	77±5
4	**14e**	3-$F_3CC_6H_4$	H	241±27
5	**14f**	H	CO_2Me	714±53
6	**14h**	H	CONHMe	595±80

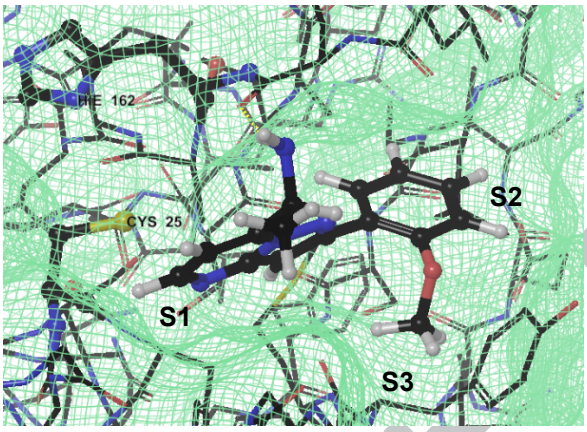
[a] Determined by non-linear regression analysis using GraphPad Prism 5.0 with the model for linear competitive inhibition.

All pyrazolo[1,5-*a*]pyrimidines **10**, **11**, and **13**–**17** were subjected to molecular docking into the active site of cathepsin K [47]. The compounds bound preferentially into the cleft forming the non-primed sites (sites S1 through S3) which is the narrowest part of the active site. At the same time, site S2 which forms a well-defined pocket is also the primary specificity determinant in cathepsin K and other related proteases. Specifically, cathepsin K shows preference for hydrophobic residues as well as Pro at the corresponding P2 position of the substrate [48]. The binding modes of compounds **14**, which exhibited quantifiable inhibitory activity, are shown in Figure 3. In all cases, the S2 pocket was occupied by a hydrophobic group which is in accordance with the specificity of cathepsin K. Interestingly, the binding pose of compound **14d**, which had the highest affinity for cathepsin K *in vitro*

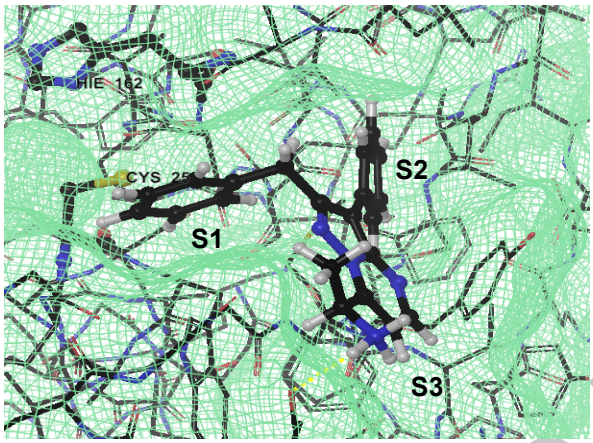
and the second-best docking score *in silico*, is the only compound that occupies all three non-primed sites.



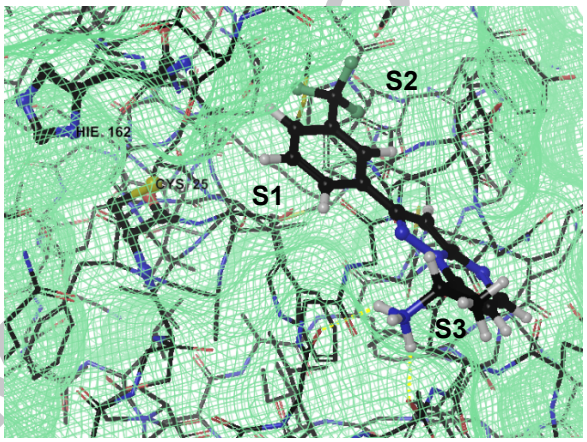
14a (–3.85)



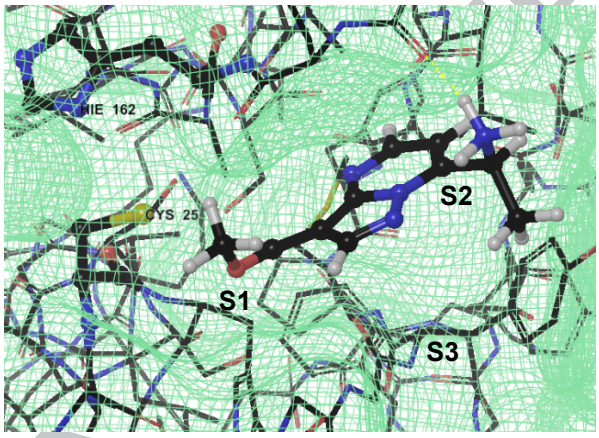
14b (–3.18)



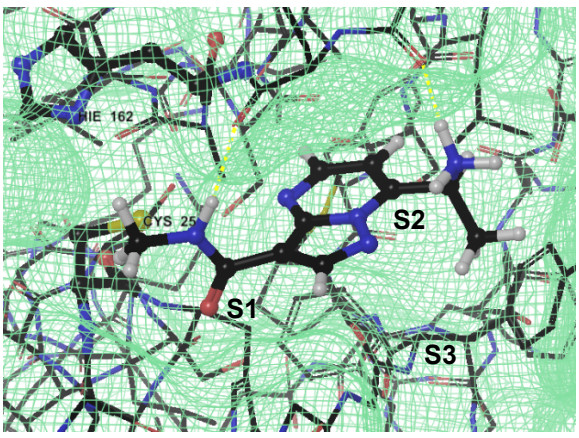
14d (–5.62)



14e (–5.60)



14f (–3.83)



14h (–4.49)

Fig. 3. Molecular docking of compounds **14a**,**b**,**d**,**e**,**f**,**h** into the active site of cathepsin K with corresponding docking scores. The S1, S2 and S3 sites of the active site are marked in the representations. Hydrogen bonds of the amino group are marked yellow.

4. Conclusions

We developed a general synthetic route towards 7-(1-aminoalkyl)pyrazolo[1,5-*a*]pyrimidine derivatives starting with regioselective cyclocondensation of 3-aminopyrazoles with aminoalkyl ethynyl ketones, which in turn are easily accessible from α-amino acids. The method has a broad scope in terms of diversity of substituents and functional groups attached to the pyrazolo[1,5-*a*]pyrimidine core. Beside this, also the degree of saturation of the heterocyclic scaffold, i.e. it's 3D character can be increased by stereoselective catalytic hydrogenation. In summary, this allows for easy preparation of structurally diverse pyrazolo[1,5-*a*]pyrimidine derivatives. The synthesised compounds were then tested for inhibition of cathepsin K. The strongest affinity was determined for the free amino compound **14d** with a K_i value of 77±5 μM. Subsequent molecular docking of the above compounds showed that the S2 pocket was occupied by a hydrophobic group which is in accordance with the specificity of cathepsin K. The binding pose of compound **14d** with the highest affinity for cathepsin K *in vitro* is the only one that occupies all three non-primed sites, S1–S3. In summary, compound **14d** could represent a lead for further development of cathepsin K inhibitors, in particular because of viability of synthetic approach that enables the preparation of structurally diverse compounds based on pyrazolo[1,5-*a*]pyrimidine scaffold.

3. Experimental

3.1. Materials and methods

Melting points were determined on a Kofler micro hot stage and on a Stanford Research Systems MPA100 OptiMelt automated melting point system. The NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 using Me_4Si as the internal standard on a Bruker Avance DPX 300 and Bruker Avance III UltraShield 500 plus instruments at 300 and 500 MHz for ^1H and at 75.5 and 126 MHz for ^{13}C nucleus, respectively. Mass spectra were recorded on Agilent 6224 Accurate Mass TOF LC/MS spectrometer and IR spectra on a Bruker FTIR Alpha Platinum spectrophotometer. Microanalyses were performed by combustion analysis on a Perkin-Elmer CHN Analyzer 2400 II. Flash column chromatography (FC), column chromatography (CC), and dry-vacuum flash chromatography (DVFC) were performed on silica gel (particle size: 35–70 μm).

Esters **1a**,**b**, nitriles **2a**,**b**, bromoketones **3a**,**b**, 3-amino-1*H*-indazole (**5h**), *N*-Boc-amino acids **6a**–**c**, and amines **12a**–**i** are commercially available. 5-Aminopyrazoles **5a**–**g** [29–32] and ynones **7a**–**c** [45] were prepared following the literature procedures.

Molecular docking was performed with Glide, a part of a Schrödinger suite software [49].

3.1.1. Enzyme assays

Kinetic measurements were performed in a 10 X 10-mm quartz cuvette at 25 °C with constant stirring on a Varian Cary 50 Bio UV/VIS spectrophotometer in a reaction volume of 3 mL of 100 mM sodium acetate buffer pH 5.50 containing 1 mM EDTA, 5 mM DTT, 20 microM of the substrate Z-FR-pNA and respective concentrations of tested compounds. The determined value of the Michaelis constant K_m for this substrate under the described experimental conditions was 16 microM. Bulk solutions of potential inhibitors were made in DMSO. Absorbance was observed for 60 s at 405 nm and initial reaction rates determined from the progress curves using linear regression. Materials used:

recombinant human cathepsin K, made in biochemistry department of UL FKKT; DTT, Sigma-Aldrich, ZDA; Z-FR-pNA, Bachem, Switzerland.

Kinetic analyses. Titration curves of cathepsin K with the tested compounds (I) in the presence of substrate (S) were analysed with the model for linear competitive inhibition in which the reaction rate in the presence of inhibitor v_i is defined by equation 1:

$$v_i = \frac{v_0\left(1 + \frac{[S]}{K_m}\right)}{1 + \frac{[S]}{K_m} + \frac{[I]}{K_i}} \qquad \text{Eq. 1}$$

where v_o is the reaction rate in the absence of inhibitor and Ki is the inhibition constant, i.e. the equilibrium dissociation constant of the EI complex. All analyses were performed using GraphPad Prism 5.0 Software (GraphPad Software, La Jolla, CA, USA).

*3.2. Synthesis of compounds **10**.*

*3.2.1. General procedure for the synthesis of compounds **10** A (G.P.A).*

A mixture of 5-aminopyrazole **5** (1.1 mmol), ynone **7** (1 mmol), and MeOH (5 mL) was stirred at r.t. for 7 days. The product was, either collected by filtration or washed with MeOH (2 mL), or the solvent was evaporated in vacuo and the residue was purified by CC (EtOAc/hexanes). Fractions containing the product were combined and evaporated in vacuo to give **10**.

*3.2.2. General procedure for the synthesis of compounds **10** B (G.P.B).*

A solution of 5-aminopyrazole **5g** (1 mmol) and ynone **7** (1 mmol) in MeOH (1 mL) was added dropwise to a mixture of AcOH (0.5 mL) and water (10 mL) and the mixture was stirred at r.t. for 7

days. The product was extracted by CH_2Cl_2 (3 × 25 mL) and the combined organic phases were dried over anhydrous Na_2SO_4, filtered, and the filtrate was evaporated in vacuo. The residue was purified by CC (EtOAc/hexanes). Fractions containing the product were combined and evaporated in vacuo to give **10**.

*3.2.3. 7-{[(tert-Butoxycarbonyl)amino]methyl}-2-phenylpyrazolo-[1,5-a]pyrimidine (**10a**).* Prepared from **5a** (175 mg, 1.1 mmol) and **7a** (183 mg, 1.0 mmol), G.P.A, CC (EtOAc-hexanes, 1:2). Yellow solid (70 mg, 43%); mp 76–80 °C; ν_{max}/cm^{-1} (ATR) 3208, 2975, 1738, 1712, 1617, 1548, 1463, 1444, 1407, 1392, 1365, 1281, 1251, 1163, 1083, 1049, 944, 860, 814, 770, 742, 697, 640; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.46 (s, 9H), 4.85 (d, *J* = 6.5 Hz, 2H), 5.65 (s, 1H), 6.82 (d, *J* = 4.2 Hz, 1H), 7.03 (s, 1H), 7.39–7.45 (m, 1H), 7.46–7.51 (m, 2H), 7.99–8.05 (m, 2H), 8.45 (d, *J* = 4.2 Hz, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 28.3, 40.0, 80.4, 93.8, 105.6, 126.6, 128.8, 129.1, 132.7, 145.0, 149.2, 150.0, 155.7, 155.9; HRMS (ESI): MH^+, found 325.1659. $[C_{18}H_{21}N_4O_2]^+$ requires 325.1659.

*3.2.4. Methyl 7-{[(tert-butoxycarbonyl)amino]methyl}pyrazolo[1,5-a]pyrimidine-3-carboxylate (**10b**).* Prepared from **5g** (706 mg, 5.0 mmol) and **7a** (915 mg, 5.0 mmol), G.P.B, CC (EtOAc-hexanes, 1:1). White solid (1.13 g, 74%); mp 160–164 °C; ν_{max}/cm^{-1} (ATR) 3260, 2965, 1703, 1618, 1547, 1531, 1475, 1432, 1400, 1385, 1364, 1354, 1281, 1246, 1229, 1167, 1085, 1050, 1030, 930, 894, 867, 834, 803, 783, 745, 722, 667, 642, 626; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.45 (s, 9H), 3.98 (s, 3H), 4.83 (d, *J* = 6.5 Hz, 2H), 5.51 (s, 1H), 7.04 (d, *J* = 4.3 Hz, 1H), 8.61 (s, 1H), 8.77 (d, *J* = 4.3 Hz, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 28.3, 40.0, 51.8, 80.7, 103.2, 107.5, 146.8, 147.7, 147.8, 152.6, 155.6, 163.0; HRMS (ESI): MH^+, found 307.1396. $[C_{14}H_{19}N_4O_4]^+$ requires 307.1401; (Found: C, 54.98; H, 5.94; N, 18.23. $C_{14}H_{18}N_4O_4$ requires C, 54.89; H, 5.92; N, 18.29%).

*3.3.5. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}-2-phenylpyrazolo[1,5-a]pyrimidine (**10c**).* Prepared from **5a** (175 mg, 1.1 mmol) and **7b** (197 mg, 1.0 mmol), G.P.A, CC (EtOAc-hexanes, 1:1). Pale brown solid (319 mg, 93%); mp 127–133 °C; $[\alpha]_D^{22}$ –64.6 (c 0.17, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3345, 3114, 1680, 1614, 1525, 1463, 1443, 1391, 1366, 1331, 1306, 1269, 1248, 1219, 1162, 1112, 1080, 1059, 1021, 937, 919, 866, 846, 822, 812, 766, 747, 687, 658, 640; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.43 (s, 9H), 1.75 (d, *J* = 7.1 Hz, 3H), 5.36–5.49 (m, 1H), 5.97 (s, 1H), 6.75 (d, *J* = 4.2 Hz, 1H), 7.00 (s, 1H), 7.39–7.44 (m, 1H), 7.48 (dd, *J* = 6.7, 8.3 Hz, 2H), 8.00–8.06 (m, 2H), 8.42 (d, *J* = 4.2 Hz, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.7, 28.4, 48.0, 80.2, 93.5, 104.5, 126.5, 126.7, 128.8, 129.1, 132.8, 149.5, 150.5, 154.9, 155.7; HRMS (ESI): MH^+, found 339.1814. $[C_{19}H_{23}N_4O_2]^+$ requires 339.1816; (Found: C, 66.93; H, 6.88; N, 16.00. $C_{19}H_{23}N_4O_2 \cdot \frac{1}{5}H_2O$ requires C, 66.73; H, 6.60; N, 16.38%).

*3.3.6. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}-2-(2-methoxyphenyl)pyrazolo[1,5-a]pyrimidine (**10d**).* Prepared from **5b** (208 mg, 1.1 mmol) and **7b** (197 mg, 1.0 mmol), G.P.A, CC (EtOAc-hexanes, 1:1). Pale yellow solid (316 mg, 86%); mp 124–126 °C; $[\alpha]_D^{22}$ –71.1 (c 0.19, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3370, 2974, 1678, 1613, 1583, 1538, 1516, 1479, 1443, 1392, 1369, 1331, 1304, 1247, 1161, 1114, 1060, 1020, 938, 862, 848, 822, 797, 783, 746, 689, 658, 637; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.42 (s, 9H), 1.75 (d, J = 7.1 Hz, 3H), 3.97 (s, 3H), 5.32–5.48 (m, 1H), 6.11 (s, 1H), 6.72 (d, J = 4.2 Hz, 1H), 7.05 (dd, J = 8.4, 1.0 Hz, 1H), 7.09 (td, J = 7.5, 1.1 Hz, 1H), 7.29 (s, 1H), 7.40 (ddd, J = 8.3, 7.4, 1.8 Hz, 1H), 8.15 (dd, J = 7.8, 1.8 Hz, 1H), 8.41 (d, J = 4.2 Hz, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.7, 28.4, 48.2, 55.6, 80.1, 97.9, 104.4, 111.5, 120.8, 121.7, 129.4, 130.1, 148.1, 148.9, 150.0, 152.7, 154.9, 157.6; HRMS (ESI): MH^+, found 369.1921. $[C_{20}H_{25}N_4O_3]^+$ requires 369.1920.

*3.3.7. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}-2-methyl-3-phenylpyrazolo[1,5-a]pyrimidin-7-yl)ethyl]carbamate (**10e**).* Prepared from **5c** (191 mg, 1.1 mmol) and **7b** (197 mg, 1.0

mmol), G.P.A, CC (EtOAc-hexanes, 1:4). Yellow solid (210 mg, 60%); mp 143–146 °C; $[\alpha]_D^{22}$ –60.5 (c 0.20, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3357, 3983, 1678, 1608, 1552, 1521, 1476, 1441, 1391, 1367, 1345, 1325, 1302, 1253, 1208, 1164, 1115, 1077, 1062, 1029, 1007, 908, 863, 825, 773, 748, 697, 652, 633; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.44 (s, 9H), 1.70 (d, *J* = 7.1 Hz, 3H), 2.65 (s, 3H), 5.32–5.43 (m, 1H), 6.01 (s, 1H), 6.72 (d, *J* = 4.2 Hz, 1H), 7.27–7.35 (m, 1H), 7.48 (t, *J* = 7.7 Hz, 2H), 7.62–7.75 (m, 2H), 8.43 (d, *J* = 4.2 Hz, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 14.3, 18.8, 28.4, 47.8, 80.1, 104.1, 109.4, 126.4, 128.6, 128.9, 132.3, 146.8, 148.5, 149.3, 152.2, 154.9; HRMS (ESI): MH^+, found 353.1973. $[C_{20}H_{25}N_4O_2]^+$ requires 353.1972; (Found: C, 68.32; H, 6.80; N, 15.88. $C_{20}H_{24}N_4O_2$ requires C, 68.16; H, 6.86; N, 15.90%).

*3.3.8. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}-2-benzyl-3-phenylpyrazolo[1,5-a]pyrimidin-7-yl)ethyl]carbamate (**10f**).* Prepared from **5d** (274 mg, 1.1 mmol) and **7b** (197 mg, 1.0 mmol), G.P.A, CC (EtOAc-hexanes, 1:4). Pale yellow solid (253 mg, 59%); mp 131–139 °C; $[\alpha]_D^{22}$ –48.0 (c 0.18, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3351, 2981, 1677, 1611, 1556, 1522, 1493, 1442, 1391, 1366, 1328, 1297, 1265, 1249, 1213, 1161, 1112, 1074, 1060, 1027, 1007, 859, 829, 779, 755, 737, 720, 694, 662, 625, 606; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.43 (s, 9H), 1.70 (d, *J* = 7.0 Hz, 3H), 4.33 (d, *J* = 1.4 Hz, 2H), 5.35 (dq, *J* = 8.3, 8.8 Hz, 1H), 6.74 (d, *J* = 4.2 Hz, 1H), 6.05 (s, 1H), 7.18–7.55 (m, 10H), 8.44 (d, *J* = 4.2 Hz, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.8, 28.4, 33.8, 48.1, 80.1, 104.6, 110.0, 126.2, 126.7, 128.4, 128.6, 128.7, 129.4, 131.9, 139.3, 146.9, 148.4, 149.4, 154.1, 154.9; HRMS (ESI): MH^+, found 429.2283. $[C_{26}H_{29}N_4O_2]^+$ requires 429.2285; (Found: C, 72.32; H, 6.44; N, 12.97. $C_{26}H_{28}N_4O_2 \cdot ¼H_2O$ requires C, 72.11; H, 6.63; N, 12.94%).

*3.3.9. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}-2-(3-nitrophenyl)pyrazolo[1,5-a]pyrimidine (**10g**).* Prepared from **5e** (225 mg, 1.1 mmol) and **7b** (197 mg, 1.0 mmol), G.P.A, isolated

by filtration. Pale yellow solid (219 mg, 57%); mp 218–223 °C; $[\alpha]_D^{22}$ –30.3 (c 0.15, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3350, 2979, 1679, 1611, 1526, 1464, 1394, 1368, 1344, 1328, 1303, 1268, 1251, 1218, 1164, 1113, 1061, 1023, 957, 815, 864, 845, 800, 777, 761, 743, 698, 659, 636; δ_H (500 MHz; DMSO-d_6; Me_4Si) 1.40 (s, 9H), 1.54 (d, *J* = 7.1 Hz, 3H), 5.49 (p, *J* = 7.1 Hz, 1H), 7.00 (d, *J* = 4.4 Hz, 1H), 7.52 (s, 1H), 7.83 (t, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 7.7 Hz, 1H), 8.29 (ddd, *J* = 8.2, 2.4, 1.0 Hz, 1H), 8.54 (dt, *J* = 7.8, 1.3 Hz, 1H), 8.61 (d, *J* = 4.3 Hz, 1H), 8.86 (t, *J* = 2.0 Hz, 1H); δ_C (126 MHz; DMSO-d6; Me_4Si) 18.3, 28.6, 45.6, 79.1, 94.6, 104.6, 120.8, 124.1, 131.1, 133.1, 134.7, 148.9, 150.3, 150.9, 152.3, 152.9, 155.5; HRMS (ESI): MH^+, found 384.1661. $[C_{19}H_{22}N_5O_4]^+$ requires 384.1666; (Found: C, 58.83; H, 5.34; N, 17.79. $C_{19}H_{21}N_5O_4 \cdot ¼H_2O$ requires C, 58.83; H, 5.59; N, 18.05%).

*3.3.10. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}-2-(3-trifluorophenyl)pyrazolo[1,5-a]pyrimidine (**10h**).* Prepared from **5f** (250 mg, 1.1 mmol) and **7b** (197 mg, 1.0 mmol), G.P.A, isolation by filtration. White solid (303 mg, 75%); mp 196–197 °C; $[\alpha]_D^{22}$ –33.2 (c 0.16, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3351, 2990, 1679, 1617, 1524, 1466, 1450, 1395, 1369, 1331, 1303, 1270, 1253, 1226, 1158, 1112, 1100, 1080, 1060, 1021, 951, 917, 853, 824, 798, 782, 749, 694, 657, 635; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.44 (s, 9H), 1.74 (d, *J* = 7.1 Hz, 3H), 5.48 (p, *J* = 7.2 Hz, 1H), 5.66–5.86 (m, 1H), 6.81 (d, *J* = 4.2 Hz, 1H), 7.04 (s, 1H), 7.60 (t, *J* = 7.7 Hz, 1H), 7.66 (d, *J* = 7.8 Hz, 1H), 8.19 (d, *J* = 7.7 Hz, 1H), 8.26 (d, *J* = 2.2 Hz, 1H), 8.46 (d, *J* = 4.2 Hz, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.7, 28.3, 47.6, 80.4, 93.8, 104.6, 123.4 (q, *J* = 3.8 Hz), 124.1 (q, *J* = 273.4 Hz), 125.5 (q, *J* = 3.8 Hz), 129.3, 129.9, 131.2 (q, *J* = 32.7 Hz), 133.7, 149.5, 149.7, 150.6, 164.2, 154.8; HRMS (ESI): MH^+, found 407.1689. $[C_{20}H_{22}F_3N_4O_2]^+$ requires 407.1683; (Found: C, 59.20; H, 4.99; N, 13.82. $C_{20}H_{21}F_3N_4O_2$ requires C, 59.11; H, 5.21; N, 13.79%).

*3.3.11. Methyl (1'S)-7-{1-[(tert-butoxycarbonyl)amino]ethyl}pyrazolo[1,5-a]pyrimidine-3-carboxylate (**10i**).* Prepared from **5g** (1.425 g, 10.1 mmol) and **7b** (2.00 g, 10.1 mmol), G.P.B, CC (EtOAc-hexanes, 2:1). Pale yellow solid (2.27 g, 70%); physical and spectral data were in agreement with the literature data [28].

*3.3.12. (4'S)-4-[1-[(tert-Butoxycarbonyl)amino]ethyl]pyrimido[1,2-b]indazole (**10j**).* Prepared from **5h** (146 mg, 1.1 mmol) and **7b** (197 mg, 1.0 mmol), G.P.A, CC (EtOAc-hexanes, 1:1). Brown solid (133 mg, 46%); mp 131–135 °C; $[\alpha]_D^{22}$ –78.6 (c 0.16, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3357, 2978, 1680, 1635, 1595, 1519, 1441, 1391, 1366, 1344, 1324, 1301, 1247, 1162, 1125, 1078, 1055, 1013, 861, 831, 752, 735, 645; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.26–1.55 (m, 9H), 1.78 (d, *J* = 7.1 Hz, 3H), 5.57 (p, *J* = 7.7 Hz, 1H), 6.16–6.38 (m, 1H), 7.13–7.26 (m, 1H), 7.31 (d, *J* = 8.2, 6.7 Hz, 1H), 7.64 (ddd, *J* = 8.3, 6.7, 1.1 Hz, 1H), 7.87 (d, *J* = 8.6 Hz, 1H), 8.33 (d, *J* = 8.3 Hz, 1H), 8.62 (d, *J* = 4.4 Hz, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.2, 28.3, 48.4, 80.2, 108.4, 113.3, 116.3, 120.8, 120.9, 130.0, 144.5, 145.5, 147.6, 150.9, 154.9; HRMS (ESI): MH^+, found 313.1659. $[C_{17}H_{21}N_4O_2]^+$ requires 313.1654; (Found: C, 64.33; H, 6.52; N, 17.37. $C_{17}H_{20}N_4O_2 \cdot ¼H_2O$ requires C, 64.44; H, 6.52; N, 17.68%).

*3.3.13. (1'S)-7-({1-[(tert-Butoxycarbonyl)amino]-2-phenyl}ethyl)-2-phenyl-1-(2-phenylpyrazolo[1,5-a]pyrimidine (**10k**).* Prepared from **5a** (175 mg, 1.1 mmol) and **7c** (274 mg, 1.0 mmol), G.P.A, CC (EtOAc-hexanes, 1:2). Pale yellow solid (200 mg, 48%); mp 139–142 °C; $[\alpha]_D^{22}$ +24.5 (c 0.13, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3351, 2978, 1680, 1613, 1543, 1513, 1462, 1443, 1391, 1365, 1318, 1268, 1250, 1222, 1163, 1084, 1052, 1020, 938, 918, 889, 851, 817, 788, 764, 732, 691, 636; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.40 (s, 9H), 3.37 (dd, *J* = 13.6 Hz, 7.2 Hz, 1H), 3.63 (dd, *J* = 14.2 Hz, 8.3 Hz, 1H), 5.48–5.59 (m, 1H), 6.01–6.17 (m, 1H), 6.49 (d, = *J* 4.3 Hz, 1H), 7.01 (d, *J* = 6.9 Hz, 2H), 7.11

(s, 1H), 7.18–7.25 (m, 3H), 7.44–7.48 (m, 1H), 7.53 (dd, *J* = 8.3, 6.7 Hz, 2H), 8.06–8.10 (m, 2H) 8.31 (d, *J* = 4.3 Hz, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 28.3, 38.1, 53.9, 80.3, 93.6, 106.2, 126.7, 127.0, 128.6, 128.8, 129.0, 129.2, 132.7, 136.6, 146.4, 149.1, 150.4, 155.0, 155.8; HRMS (ESI): MH^+, found 415.2124. $[C_{25}H_{27}N_4O_2]^+$ requires 415.2129.

*3.3.14. Methyl (1'S)-7-{1-[(tert-butoxycarbonyl)amino]-2-phenylethyl}pyrazolo[1,5-a]pyrimidine-3-carboxylate (**10l**).* Prepared from **5g** (706 mg, 5.0 mmol) and **7c** (1.365 g, 5.0 mmol), G.P.B, CC (EtOAc-hexanes, 1:1). Pale yellow solid (400 mg, 20%); mp 143–146 °C; $[\alpha]_D^{22}$ +36.1 (c 0.23, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3336, 2974, 1704, 1683, 1620, 1553, 1526, 1487, 1455, 1436, 1377, 1336, 1317, 1276, 1255, 1219, 1199, 1161, 1100, 1049, 1031, 1001, 951, 910, 891, 850, 823, 800, 781, 755, 728, 702, 683, 642, 627; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.39 (s, 9H), 3.30 (dd, *J* = 13.7 Hz, 7.3 Hz, 1H), 3.49 (dd, *J* = 13.7 Hz, 7.6 Hz, 1H), 3.99 (s, 3H), 5.52 (q, *J* = 8.0 Hz, 1H), 6.02 (d, *J* = 9.0 Hz, 1H), 6.68 (d, *J* = 4.4 Hz, 1H), 6.90–7.00 (m, 2H), 7.13-7.25 (m, 3H), 8.60 (d, *J* = 4.4 Hz, 1H), 8.68 (s, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 28.3, 38.2, 51.8, 53.7, 80.5, 102.9, 108.1, 127.2, 128.7, 128.9, 136.0, 147.5, 148.2, 148.5, 152.5, 154.9, 163.0; HRMS (ESI): MH^+, found 397.187 $[C_{21}H_{25}N_4O_4]^+$ requires 397.187; (Found: C, 63.42; H, 6.08; N, 13.81. $C_{21}H_{24}N_4O_4 \cdot ⅛\ H_2O$ requires C, 63.26; H, 6.13; N, 14.05%).

*3.4. General procedure for the synthesis of compounds **11**.*

A mixture of the ester **10** (0.5 mmol), MeOH (7 mL), water (5 mL), and $LiOH \cdot H_2O$ (46 mg, 1.1 mmol) was stirred at r.t. for 4 days. Saturated aq. $NaHCO_3$ (20 mL) was added, the non-polar impurities were extracted with CH_2Cl_2 (3 × 10 mL) and the aqueous phase was acidified with 1 M aq.

$NaHSO_4$ (15 mL). The product was extracted with CH_2Cl_2 (3 × 15 mL), the combined organic phases were dried over anhydrous Na_2SO_4, filtered, and the filtrate was evaporated in vacuo to give **11**.

*3.4.1. 7-{[(tert-Butoxycarbonyl)amino]methyl}pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (**11a**).* Prepared from 10b (148 mg, 0.5 mmol) and $LiOH·H_2O$ following general procedure. Pale pink solid (92 mg, 65%); mp 113–141 °C (decomp.); ν_{max}/cm^{-1} (ATR) 2977, 1683, 1620, 1554, 1531, 1410, 1392, 1367, 1284, 1251, 1158, 1095, 938, 861, 821, 785, 761, 668, 637, 614; δ_H (500 MHz; DMSO-d_6; Me_4Si) 1.44 (s, 9H), 4.68 (d, J = 5.2 Hz, 2H), 7.08 (d, J = 4.4 Hz, 1H), 7.76 (t, J = 6.0 Hz, 1H), 8.65 (s, 1H), 8.80 (d, J = 4.4 Hz, 1H), 12.38 (s, 1H); δ_C (126 MHz; DMSO-d_6; Me_4Si) 28.6, 55.9, 79.3, 103.2, 106.8, 147.7, 147.8, 149.0, 153.1, 156.3, 163.7; HRMS (ESI): MH^+, found 293.1240. $[C_{13}H_{17}N_4O_4]^+$ requires 293.1244.

*3.4.2. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (**11b**).* Prepared from **10i** (1.10 g, 3.4 mmol) and $LiOH·H_2O$ following general procedure. Pale yellow solid (930 mg, 89%); mp 77–80 °C; $[\alpha]_D^{22}$ –25.0 (c 0.08, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3307, 2978, 1685, 1618, 1547, 1520, 1452, 1392, 1366, 1323, 1250, 1162, 1108, 1058, 1010, 953, 909, 862, 836, 786, 756, 657, 632, 614; δ_H (500 MHz; DMSO-d_6; Me_4Si) 1.39 (s, 9H); 1.48 (d, J = 7.1 Hz, 3H); 5.41 (p, J = 7.1 Hz, 1H); 7.16 (d, J = 4.4 Hz, 1H); 7.97 (d, J = 7.5 Hz, 1H); 8.67 (s, 1H); 8.83 (d, J = 4.3 Hz, 1H); 12.45 (s, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.4, 28.6, 45.8, 79.2, 103.2, 105.6, 147.8, 147.9, 153.4, 153.8, 155.4, 163.6; HRMS (ESI): MH^+, found 307.1401. $[C_{14}H_{19}N_4O_4]^+$ requires 307.1401.

*3.4.3. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]-2-phenylethyl}pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (**11c**).* Prepared from **10l** (198 mg, 0.5 mmol) and $LiOH·H_2O$ following general

procedure. Pale brown solid (103 mg, 54%); mp 86–95 °C (decomp.); $[\alpha]_D^{22}$ –20.5 (c 0.14, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3340, 2978, 1683, 1619, 1548, 1520, 1494, 1454, 1392, 1366, 1274, 1250, 1160, 1106, 1048, 1017, 919, 889, 852, 821, 780, 750, 730, 698, 645; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.39 (s, 9H), 3.31 (dd, *J* = 13.8 Hz, 7.4 Hz, 1H), 3.50 (dd, *J* = 13.6 Hz, 7.9 Hz, 1H), 5.54 (d, *J* = 8.4 Hz, 1H), 5.88 (d, *J* = 8.8 Hz, 1H), 6.74 (s, 1H), 6.97 (d, *J* = 6.4 Hz, 2H), 7.18–7.31 (m, 3H), 8.57 (s, 1H), 8.74 (s, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 28.3, 38.1, 53.7, 80.7, 102.4, 108.2, 127.3, 128.8, 128.9, 135.8, 147.6, 148.4, 149.4, 152.4, 154.9, 165.3; HRMS (ESI): MH^+, found 383.1713. $[C_{20}H_{23}N_4O_4]^+$ requires 383.1714.

*3.5. General procedure for the synthesis of compounds **13**.*

Under Ar CDI (0.5 mmol) was added to a solution of **11** (0.4 mmol) in anhydrous THF (5 mL) and the mixture was stirred at r.t. for 2 h. Amine **12** (2.0 mmol) or its respective salt (0.44 mmol) and triethylamine (0.44 mmol) was added and stirring at r.t. was continued for 16 h. The mixture was acidified with 1 M aq. $NaHSO_4$ (10 mL) and the product was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic phases are washed with saturated $NaHCO_3$ (10 mL), dried over anhydrous Na_2SO_4, filtered, and the filtrate was evaporated in vacuo. The residue was purified by CC (EtOAc/hexanes). Fractions containing the product were combined and evaporated in vacuo to give **13**.

*3.5.1. 7-[(tert-Butoxycarbonyl)aminomethyl]pyrazolo[1,5-a]pyrimidine-3-(N-methylcarboxamide) (**13a**).*

Prepared from **11b** (65 mg, 0.22 mmol), **12a**·HCl (8 mg, 0.24 mmol), and triethylamine (34 μL, 0.24 mmol) following general procedure. Yellow solid (33 mg, 49%); mp 168–170 °C; ν_{max}/cm^{-1} (ATR) 3308, 2931, 1718, 1635, 1618, 1557, 1511, 1415, 1365, 1329, 1288, 1250, 1158, 1126, 1094, 1053, 968, 938, 906, 869, 845, 802, 779, 659, 643, 620; δ_H (500 MHz; $CDCl_3$;

Me_4Si) 1.45 (s, 9H), 3.07 (d, J = 4.9 Hz, 3H), 4.82 (d, J = 6.5 Hz, 2H), 5.58 (s, 1H), 7.00 (d, J = 4.3 Hz, 1H), 7.89 (d, J = 5.4 Hz, 1H), 8.59 (d, J = 4.3 Hz, 1H), 8.68 (s, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 25.9, 28.3, 40.0, 80.7, 106.3, 106.8, 146.0, 146.4, 147.0, 150.6, 155.7, 162.7; HRMS (ESI): MH^+, found 306.1556. $[C_{14}H_{20}N_5O_3]^+$ requires 306.1561.

*3.5.2. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}pyrazolo[1,5-a]pyrimidine-3-(N-methylcarboxamide) (**13b**).* Prepared from **11i** (122 mg, 0.4 mmol), **12a**·HCl (14 mg, 0.44 mmol), and trimethylamine (62 μL, 0.44 mmol) following general procedure, CC (EtOAc-hexanes, 5:1). White solid (89 mg, 70%); mp 157–161 °C; $[\alpha]_D^{22}$ –27.6 (c 0.19, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3735, 3348, 3088, 2979, 2185, 2167, 1868, 1680, 1642, 1617, 1564, 1548, 1524, 1447, 1406, 1366, 1328, 1304, 1264, 1250, 1161, 1126, 1103, 1060, 1016, 939, 863, 828, 812, 781, 669, 652, 632; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.42 (s, 9H), 1.69 (d, J = 7.1 Hz, 3H), 3.07 (d, J = 4.8 Hz, 3H), 5.40 (p, J = 7.5 Hz, 1H), 5.81 (s, 1H), 6.94 (d, J = 4.3 Hz, 1H), 7.91 (q, J = 5.0 Hz, 1H), 8.56 (d, J = 4.3 Hz, 1H), 8.69 (s, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.6, 25.9, 28.3, 47.8, 80.4, 105.4, 106.0, 146.3, 146.5, 150.8, 151.1, 154.8, 162.7; HRMS (ESI): MH^+, found 320.1718. $[C_{15}H_{22}N_5O_3]^+$ requires 320.1717; (Found: C, 56.59; H, 6.52; N, 21.93. $C_{15}H_{21}N_5O_3$ requires C, 56.41; H, 6.63; N, 21.93%).

*3.5.3. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}pyrazolo[1,5-a]pyrimidine-3-(N-propylcarboxamide) (**13c**).* Prepared from **11i** (122 mg, 0.4 mmol) and **12b** (164 μL, 2.0 mmol), following general procedure, CC (EtOAc-hexanes, 2:1). White solid (110 mg, 87%); mp 66–74 °C; $[\alpha]_D^{22}$ –27.1 (c 0.18, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3277, 2966, 2932, 2875, 2168, 2059, 1997, 1943, 1713, 1640, 1551, 1526, 1454, 1365, 1326, 1282, 1248, 1162, 1126, 1060, 1008, 905, 862, 834, 779, 760, 666, 635; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.02 (t, J = 7.4 Hz, 3H), 1.42 (s, 9H), 1.60–1.74 (m, 5H), 3.48 (dt, J = 7.7, 6.3 Hz, 2H), 5.41 (q, J = 7.3 Hz, 1H), 5.76–5.95 (m, 1H), 6.95 (d, J = 4.3 Hz, 1H), 8.01 (t,

J = 5.8 Hz, 1H), 8.58 (d, *J* = 4.3 Hz, 1H), 8.69 (s, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 11.5, 18.6, 23.1, 28.3, 40.8, 47.7, 80.4, 105.4, 106.1, 146.4, 146.5, 150.8, 151.1, 154.8, 162.1; HRMS (ESI): MH^+, found 348.2026. $[C_{17}H_{26}N_5O_3]^+$ requires 348.2030; (Found: C, 58.57; H, 7.00; N, 19.97. $C_{17}H_{25}N_5O_3$ requires C, 58.77; H, 7.25; N, 20.16%).

*3.5.4. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}pyrazolo[1,5-a]pyrimidine-3-(N-benzylcarboxamide) (**13d**).* Prepared from **11i** (122 mg, 0.4 mmol) and **12c** (218 μL, 2.0 mmol) following general procedure, CC (EtOAc-hexanes, 1:2 and 2:1). White solid (160 mg, 100%); mp 184–190 °C; $[\alpha]_D^{22}$ –24.7 (c 0.18, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3327, 3253, 2977, 1711, 1647, 1613, 1583, 1537, 1495, 1449, 1426, 1388, 1361, 1322, 1280, 1251, 1212, 1165, 1130, 1108, 1075, 1060, 1031, 1007, 946, 921, 896, 867, 836, 794, 779, 763, 726, 696, 663, 641, 624; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.42 (s, 9H), 1.68 (d, *J* = 7.1 Hz, 3H), 4.74 (dd, *J* = 6.0, 2.9 Hz, 2H), 5.41 (q, *J* = 7.3 Hz, 1H), 5.70–5.92 (m, 1H), 6.93 (d, *J* = 4.4 Hz, 1H), 7.23–7.29 (m, 1H), 7.34 (dd, *J* = 8.4, 6.7 Hz, 2H), 7.40 (d, *J* = 7.1 Hz, 2H), 8.36 (t, *J* = 6.0 Hz, 1H), 8.53 (d, *J* = 4.4 Hz, 1H), 8.73 (s, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.6, 28.3, 43.0, 47.8, 80.5, 105.5, 105.8, 127.2, 127.6, 128.6, 139.0, 146.5, 146.6, 151.0, 151.1, 154.8, 162.1; HRMS (ESI): MH^+, found 396.2033. $[C_{21}H_{26}N_5O_3]^+$ requires 396.2030.

*3.5.5. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}pyrazolo[1,5-a]pyrimidine-3-[N-(3-hydroxypropyl)carboxamide] (**13e**).* Prepared from **11i** (122 mg, 0.4 mmol) and **12d** (152 μL, 2.0 mmol) following general procedure. White solid (130 mg, 89%); mp 177–183 °C; $[\alpha]_D^{22}$ –7.6 (c 0.13, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3328, 3224, 1705, 1638, 1563, 1545, 1530, 1480, 1455, 1439, 1390, 1366, 1323, 1298, 1266, 1247, 1169, 1107, 1060, 1003, 936, 863, 840, 921, 783, 760, 714, 670, 630; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.42 (s, 9H), 1.69 (d, *J* = 7.1 Hz, 3H), 1.81 (p, *J* = 5.8 Hz, 2H), 3.69 (h, *J* = 5.3, 4H), 3.86 (d, *J* = 6.6 Hz, 1H), 5.42 (p, *J* = 7.2 Hz, 1H), 5.84 (d, *J* = 7.9 Hz 1H), 6.98 (d, *J* = 4.4 Hz,

1H), 8.17 (t, *J* = 6.4 Hz 1H), 8.59 (d, *J* = 4.4 Hz, 1H), 8.68 (s, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.5, 28.3, 33.1, 35.1, 47.7, 58.6, 80.5, 105.3, 105.6, 146.4, 146.6, 151.1, 151.4, 154.8, 163.6; HRMS (ESI): MH^+, found 364.1979. $[C_{17}H_{26}N_5O_4]^+$ requires 364.1979; (Found: C, 55.60; H, 7.11; N, 18.72. $C_{17}H_{25}N_5O_4 \cdot ⅓H2O$ requires C, 56.27; H, 7.00; N, 18.96%).

*3.5.6. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}pyrazolo[1,5-a]pyrimidine-3-(N-pentadecylcarboxamide) (**13f**).* Prepared from **11i** (122 mg, 0.4 mmol), **12e**·HCl (100 mg, 0.44 mmol), and trimethylamine (62 μL, 0.44 mmol) following general procedure. Pale yellow waxy solid (125 mg, 60%); mp 73–78 °C; $[\alpha]_D^{22}$ –16.0 (c 0.29, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3358, 2920, 2849, 1698, 1642, 1614, 1552, 1525, 1499, 1467, 1385, 1366, 1326, 1291, 1280, 1249, 1162, 1126, 1069, 1007, 907, 861, 823, 779, 762, 722, 660, 641; δ_H (500 MHz; $CDCl_3$; Me_4Si) 0.88 (t, *J* = 6.9 Hz, 3H), 1.25 (s, 24H), 1.45 (s, 9H), 1.64 (q, *J* = 7.4 Hz, 2H), 1.69 (d, *J* = 6.9 Hz, 3H), 3.50 (q, *J* = 6.7 Hz, 2H), 5.40 (p, *J* = 7.1 Hz, 1H), 5.85 (d, *J* = 8.0 Hz 1H), 6.94 (d, *J* = 4.4 Hz, 1H), 7.97 (t, *J* = 5.8 Hz 1H), 8.57 (d, *J* = 4.4 Hz, 1H), 8.69 (s, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 14.1, 18.6, 22.7, 27.1, 28.3, 29.4, 29.4, 29.6, 29.6, 29.7, 29.7, 29.7, 29.9, 31.9, 39.1, 47.8, 80.4, 105.4, 106.1, 146.4, 146.5, 150.8, 151.0, 154.8, 162.0; HRMS (ESI): MH^+, found 516.3903. $[C_{29}H_{50}N_5O_3]^+$ requires 516.3908.

*3.5.7. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}-3-[(pyrrolidin-1-yl)carbonyl]pyrazolo[1,5-a]pyrimidine (**13g**).* Prepared from **11i** (122 mg, 0.4 mmol) and **12f** (164 μL, 2.0 mmol) following general procedure. Pale yellow solid (130 mg, 90%); mp 165–188 °C; $[\alpha]_D^{22}$ –14.3 (c 0.21, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3297, 2963, 2876, 1712, 1624, 1604, 1562, 1542, 1523, 1487, 1435, 1391, 1367, 1345, 1331, 1308, 1295, 1282, 1250, 1161, 1125, 1114, 1058, 1012, 973, 829, 899, 864, 845, 827, 775, 734, 710, 688, 655, 610; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.43 (s, 9H), 1.67 (d, *J* = 7.0 Hz, 3H), 1.93 (q, *J* = 6.5 Hz, 2H), 1.99 (q, *J* = 6.5 Hz, 2H), 3.60–3.80 (m, 4H), 5.39 (p, *J* = 7.3 Hz,

1H), 5.86 (d, *J* = 8.3 Hz 1H), 6.88 (d, *J* = 4.3 Hz, 1H), 8.43 (s, 1H), 8.57 (d, *J* = 4.2 Hz, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.6, 24.5, 26.2, 28.3, 46.3, 47.8, 48.5, 80.3, 105.1, 108.2, 145.3, 146.0, 150.2, 150.7, 154.8, 162.8; HRMS (ESI): MH^+, found 360.2027. $[C_{18}H_{26}N_5O_3]^+$ requires 360.2030; (Found: C, 60.20; H, 7.26; N, 19.06. $C_{18}H_{25}N_5O_3 \cdot {}^1/_{16}H_2O$ requires C, 59.96; H, 7.02; N, 19.42%).

*3.5.8. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}-3-[(piperidin-1-yl)carbonyl]pyrazolo[1,5-a]pyrimidine (**13h**).* Prepared from **11i** (122 mg, 0.4 mmol) and **12g** (197 µL, 2.0 mmol) following general procedure. Pale yellow solid (148 mg, 99%); mp 152–179 °C; $[\alpha]_D^{22}$ –7.1 (c 0.21, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 1714, 1620, 1605, 1561, 1522, 1490, 1436, 1391, 1366, 1332, 1294, 1251, 1227, 1162, 1125, 1109, 1094, 1056, 1001, 954, 899, 865, 842, 821, 776, 762, 716, 670, 647; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.43 (s, 9H), 1.59–1.73 (m, 9H), 3.44–3.86 (m, 4H), 5.38 (p, *J* = 7.3 Hz, 1H), 5.85 (d, *J* = 8.3 Hz, 1H), 6.87 (d, *J* = 4.2 Hz, 1H), 8.38 (s, 1H), 8.56 (d, *J* = 4.3 Hz, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.6, 24.7, 25.6, 26.8, 28.3, 43.5, 47.7, 48.9, 80.3, 105.0, 106.9, 145.7, 145.7, 150.1, 150.8, 154.8, 163.0; HRMS (ESI): MH^+, found 374.2189. $[C_{19}H_{28}N_5O_3]^+$ requires 374.2187; (Found: C, 59.69; H, 7.00; N, 17.91. $C_{19}H_{27}N_5O_3 \cdot ½H_2O$ requires C, 59.67; H, 7.38; N, 18.31%).

*3.5.9. (1'S)-7-{1-[(tert-Butoxycarbonyl)amino]ethyl}-3-(morpholin-4-yl)carbonyl]pyrazolo[1,5-a]pyrimidine (**13i**).* Prepared from **11i** (122 mg, 0.4 mmol) and **12h** (174 µL, 2.0 mmol) following general procedure. Pale yellow solid (147 mg, 98%); mp 77–87 °C; $[\alpha]_D^{22}$ –10.4 (c 0.19, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3274, 2972, 2851, 1711, 1617, 1548, 1482, 1452, 1428, 1391, 1364, 1325, 1295, 1268, 1248, 1231, 1162, 1112, 1062, 1006, 956, 929, 882, 862, 830, 775, 760, 669, 647, 613; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.43 (s, 9H), 1.67 (d, *J* = 7.1 Hz, 3H), 3.70–3.90 (m, 8H), 5.40 (p, *J* = 7.2 Hz, 1H), 5.79 (d, *J* = 8.1 Hz 1H), 6.90 (d, *J* = 4.3 Hz, 1H), 8.44 (s, 1H), 8.58 (d, *J* = 4.3 Hz, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.6, 28.3, 47.2, 47.7, 66.6, 80.4, 105.2, 106.0, 145.5, 146.4, 150.5,

151.1, 154.8, 163.3; HRMS (ESI): MH^+, found 376.1975. $[C_{18}H_{26}N_5O_4]^+$ requires 376.1979; (Found: C, 57.23; H, 6.61; N, 18.09. $C_{18}H_{25}N_5O_4 \cdot ¼H_2O$ requires C, 56.90; H, 6.77; N, 18.43%).

*3.6. General procedure for the synthesis of compounds **14**.*

Compound **10**, **11**, or **13** (0.5 mmol) was dissolved in ethyl acetate (8 mL) and the solution was cooled to 0 °C (ice bath). Gradually, 2 M HCl–EtOAc (2.5 mL) was added, the mixture was left to warm up to room temperature, and then stirred at r.t. for 5 h. The precipitate was collected by filtration and washed with EtOAc (1 mL) and Et_2O (2 mL) to give **14**.

*3.6.1. (1'S)-7-(1-Aminoethyl)-2-phenylpyrazolo[1,5-a]pyrimidine hydrochloride (**14a**).* Prepared from **10a** (142 mg, 0.42 mmol) following general procedure. Yellow solid (102 mg, 88%); mp 149–153 °C ; $[\alpha]_D^{22}$ –28.2 (c 0.17, MeOH); ν_{max}/cm^{-1} (ATR) 2873, 2500, 2051, 1951, 1873, 1650, 1604, 1588, 1553, 1522, 1497, 1463, 1435, 1372, 1302, 1255, 1232, 1159, 1120, 1098, 1059, 1027, 1002, 940, 848, 828, 761, 726, 688, 652, 628, 617; δ_H (500 MHz; DMSO-d_6; Me_4Si) 1.77 (d, J = 6.9 Hz, 3H), 5.23 (p, J = 6.2 Hz, 1H), 7.37 (d, J = 4.3 Hz, 1H), 7.38 (s, 1H), 7.44–7.48 (m, 1H), 7.52 (dd, J = 8.2, 6.7 Hz, 2H), 8.10–8.20 (m, 2H), 8.66 (d, J = 4.3 Hz, 1H), 9.25 (d, J = 5.9 Hz, 3H); δ_C (126 MHz; DMSO-d_6; Me_4Si) 17.0, 45.3, 94.3, 106.2, 127.0, 129.3, 129.8, 132.6, 145.8, 150.0, 150.3, 155.4; HRMS (ESI): MH^+, found 239.1294. $[C_{14}H_{15}N_4]^+$ requires 239.1291.

*3.6.2. (1'S)-7-(1-Aminoethyl)-2-(2-methoxyphenyl)pyrazolo[1,5-a]pyrimidine hydrochloride (**14b**).* Prepared from **10d** (122 mg, 0.33 mmol) following general procedure. Yellow solid (45 mg, 44%); mp 151–157 °C; $[\alpha]_D^{22}$ +13.4 (c 0.14, MeOH); ν_{max}/cm^{-1} (ATR) 2798, 2516, 2051, 1943, 1869, 1646, 1587, 1550, 1511, 1477, 1440, 1420, 1378, 1306, 1252, 1180, 1159, 1116, 1043, 1021, 940, 852,

821, 795, 758, 689, 643; δ_H (500 MHz; DMSO-d6; Me_4Si) 1.76 (d, *J* = 6.9 Hz, 3H), 3.95 (s, 3H), 5.23 (p, *J* = 6.2 Hz, 1H), 7.10 (td, *J* = 7.5, 1.1 Hz, 1H), 7.21 (dd, *J* = 8.4, 1.0 Hz, 1H), 7.29 (s, 1H), 7.33 (d, *J* = 4.3 Hz, 1H), 7.45 (ddd, *J* = 8.9, 7.3, 1.8 Hz, 1H), 8.22 (dd, *J* = 7.7, 1.8 Hz, 1H), 8.65 (d, *J* = 4.3 Hz, 1H), 9.17 (d, *J* = 5.4 Hz, 3H); δ_C (126 MHz; DMSO-d6; Me_4Si) 16.3, 44.7, 55.6, 87.6, 105.5, 112.0, 120.3, 120.5, 128.7, 130.5, 144.7, 148.8, 149.2, 151.8, 157.2; HRMS (ESI): MH^+, found 269.1400. $[C_{15}H_{17}N_4O]^+$ requires 269.1397; (Found: C, 53.05; H, 5.06; N, 16.23. $C_{15}H_{16}N_4O{\cdot}2HCl$ requires C, 52.80; H, 5.32; N, 16.42%).

*3.6.3. (1'S)-7-(1-Aminoethyl)-2-methyl-3-phenylpyrazolo[1,5-a]pyrimidine hydrochloride (**14c**).* Prepared from **10e** (60 mg, 0.17 mmol) following general procedure. Yellow solid (15.5 mg, 32%); mp 151–160 °C; $[\alpha]_D^{22}$ +2.4 (c 0.15, MeOH); ν_{max}/cm^{-1} (ATR) 3058, 2701, 1649, 1605, 1588, 1561, 1531, 1507, 1472, 1442, 1389, 1370, 1352, 1318, 1272, 1255, 1227, 1212, 1182, 1156, 1098, 1079, 1029, 1007, 968, 917, 865, 845, 826, 799, 772, 753, 697, 661, 630, 613; δ_H (500 MHz; DMSO-d6; Me_4Si) 1.72 (d, *J* = 6.9 Hz, 3H), 2.63 (s, 3H), 5.19 (p, *J* = 6.1 Hz, 1H), 7.33 (ddd, *J* = 6.7, 6.0, 1.4 Hz, 2H), 7.49 (t, *J* = 7.7 Hz, 2H), 7.71–7.78 (m, 2H), 8.67 (d, *J* = 4.3 Hz, 1H), 9.11 (d, *J* = 5.2 Hz, 3H); δ_C (126 MHz; DMSO-d_6; Me_4Si) 14.7, 17.0, 45.1, 105.7, 109.2, 126.8, 128.9, 129.0, 132.1, 145.6, 146.1, 150.2, 152.0; HRMS (ESI): MH^+, found 253.1448. $[C_{15}H_{17}N_4]^+$ requires 253.1448.

*3.6.4. (1'S)-7-(1-Aminoethyl)-2-benzyl-3-phenylpyrazolo[1,5-a]pyrimidine hydrochloride (**14d**).* Prepared from **10f** (142 mg, 0.33 mmol) following general procedure. Yellow solid (24 mg, 20%); mp 163–168 °C; $[\alpha]_D^{22}$ –4.13 (c 0.15, MeOH); ν_{max}/cm^{-1} (ATR) 3379, 2723, 1619, 1607, 1587, 1566, 1546, 1507, 1496, 1443, 1375, 1328, 1310, 1271, 1225, 1188, 1156, 1114, 1072, 1033, 1010, 915, 872, 839, 767, 754, 737, 720, 691, 624, 605; δ_H (500 MHz; DMSO-d_6; Me_4Si) 1.73 (d, *J* = 6.8 Hz, 3H), 4.36 (s, 2H), 5.20 (p, *J* = 6.3 Hz, 1H), 7.14–7.34 (m, 6H), 7.37 (d, *J* = 4.4 Hz, 1H), 7.43 (t, *J* = 7.5

Hz, 2H), 7.60 (d, *J* = 7.6 Hz, 2H), 8.68 (d, *J* = 4.4 Hz, 1H), 9.12 (d, *J* = 5.5 Hz, 3H); δ_C (126 MHz; DMSO-d_6; Me_4Si) 16.5, 33.1, 44.6, 105.5, 109.2, 126.1, 126.5, 128.3, 128.3, 128.3, 128.8, 131.2, 138.7, 145.2, 145.7, 149.7, 153.4; HRMS (ESI): MH^+, found 329.1756. $[C_{21}H_{21}N_4]^+$ requires 329.1761.

*3.6.5. (1'S)-7-(1-Aminoethyl)-2-(3-trifluoromethylphenyl)pyrazolo-[1,5-a]pyrimidine hydrochloride (**14e**).* Prepared from **10h** (102 mg, 0.25 mmol) following general procedure. Yield: 87 mg (100%) of yellow solid; mp 163–170 °C; $[\alpha]_D^{22}$ +31.94 (c 0.16, MeOH); ν_{max}/cm^{-1} (ATR) 2856, 2510, 2067, 1962, 1890, 1638, 1588, 1554, 1520, 1475, 1447, 1414, 1375, 1354, 1307, 1291, 1254, 1235, 1217, 1196, 1167, 1128, 1099, 1068, 1029, 1002, 952, 898, 867, 852, 825, 808, 780, 736, 726, 693, 650, 626; δ_H (500 MHz; DMSO-d6; Me_4Si) 1.78 (d, *J* = 6.9 Hz, 3H), 5.26 (p, *J* = 6.2 Hz, 1H), 7.40 (d, *J* = 4.4 Hz, 1H), 7.58 (s, 1H), 7.78 (t, *J* = 7.9 Hz, 1H), 7.81–7.85 (m, 1H), 8.41–8.52 (m, 2H), 8.70 (d, *J* = 4.3 Hz, 1H), 9.21 (d, *J* = 5.7 Hz, 3H); δ_C (126 MHz; DMSO-d_6; Me_4Si) 16.3, 44.8, 94.5, 106.3, 122.6 (q, *J* = 4.0 Hz), 124.1 (q, *J* = 272.4 Hz), 125.7 (q, *J* = 4.3 Hz), 129.7 (q, *J* = 31.8 Hz), 130.0, 130.4, 133.1, 145.2, 149.5, 150.1, 153.2; HRMS (ESI): MH^+, found 307.1171. $[C_{15}H_{14}N_4F_3]^+$ requires 307.1172.

*3.6.6. Methyl (1'S)-7-(1-Aminoethyl)pyrazolo[1,5-a]pyrimidine-3-carboxylate hydrochloride (**14f**).* Prepared from **10i** (200 mg, 0.63 mmol) following general procedure. Pale brown solid (142 mg, 88%); mp 135–139 °C; $[\alpha]_D^{22}$ –107.0 (c 0.11, H_2O); ν_{max}/cm^{-1} (ATR) 3455, 2841, 2666, 2530, 2045, 1702, 1622, 1560, 1535, 1483, 1440, 1399, 1363, 1293, 1271, 1248, 1206, 1170, 1116, 1083, 992, 972, 935, 910, 828, 806, 786, 756, 719, 671, 632, 613; δ_H (500 MHz; DMSO-d_6; Me_4Si) 1.71 (d, *J* = 6.9 Hz, 3H), 3.85 (s, 3H), 5.40 (p, *J* = 7.1 Hz, 1H), 7.67 (d, *J* = 4.4 Hz, 1H), 8.78 (s, 1H), 8.96 (d, *J* = 4.5 Hz, 1H), 9.29 (s, 3H); δ_C (126 MHz; DMSO-d6; Me_4Si) 17.1, 45.3, 51.8, 102.8, 108.1, 147.5, 147.5, 147.6, 153.7, 162.5; HRMS (ESI): MH^+, found 211.1032. $[C_{10}H_{13}N_4O_2]^+$ requires 221.1033.

*3.6.7. (1'S)-7-(1-Aminoethyl)pyrazolo[1,5-a]pyrimidine-3-carboxylic acid hydrochloride (**14g**).* Prepared from **11i** (99 mg, 0.32 mmol) following general procedure. Off-white solid (40 mg, 51%); mp 188–193 °C; $[\alpha]_D^{22}$ –1.54 (c 0.18, MeOH); ν_{max}/cm^{-1} (ATR) 3304, 2875, 1707, 1624, 1556, 1498, 1478, 1408, 1388, 1358, 1317, 1278, 1259, 1229, 1201, 1163, 1151, 1109, 1089, 997, 925, 858, 835, 787, 763, 747, 656, 636; δ_H (500 MHz; DMSO-d_6; Me_4Si) 1.70 (d, J = 6.8 Hz, 3H), 5.21 (p, J = 6.8 Hz, 1H), 7.55 (d, J = 4.4 Hz, 1H), 8.71 (s, 1H), 8.92 (d, J = 4.4 Hz, 1H), 9.09 (s, 3H), 12.57 (1 H, s, CO_2H); δ_C (126 MHz; DMSO-d6; Me_4Si) 17.1, 45.3, 103.9, 107.8, 147.3, 147.7, 147.7, 153.3, 163.5; HRMS (ESI): MH^+, found 207.0878. $[C_9H_{11}N_4O_2]^+$ requires 207.0877.

*3.6.8. (1'S)-7-(1-Aminoethyl)pyrazolo[1,5-a]pyrimidine-3-(N-methylcarboxamide) hydrochloride (**14h**).* Prepared from **13b** (107 mg, 0.34 mmol) following general procedure. Yellow solid (60 mg, 86%); mp 149–153 °C; $[\alpha]_D^{22}$ +13.4 (c 0.14, MeOH); ν_{max}/cm^{-1} (ATR) 2798, 2516, 2051, 1943, 1869, 1646, 1587, 1550, 1511, 1477, 1440, 1420, 1378, 1306, 1252, 1180, 1159, 1116, 1043, 1021, 940, 852, 821, 795, 758, 689, 643; δ_H (500 MHz; DMSO-d_6; Me_4Si) 1.71 (d, J = 6.9 Hz, 3H), 2.89 (d, J = 4.0 Hz 3H), 5.22 (q, J = 6.2 Hz, 1H), 7.60 (d, J = 4.4 Hz, 1H), 7.94 (d, J = 5.5 Hz, 1H), 8.68 (s, 1H), 8.92 (d, J = 4.5 Hz, 1H), 9.22 (s, 3H); δ_C (126 MHz; DMSO-d6; Me_4Si) 16.5, 25.6, 44.7, 106.0, 106.8, 145.2, 145.4, 147.1, 151.8, 161.3; HRMS (ESI): MH^+, found 220.1191. $[C_{10}H_{14}N_5O]^+$ requires 220.1193.

*3.6.9. (1'S)-7-(1-Aminoethyl)pyrazolo[1,5-a]pyrimidine-3-(N-pentadecylcarboxamide) hydrochloride (**14i**).* Prepared from **13f** (300 mg, 0.58 mmol) following general procedure. White solid (164 mg, 62%); mp 189–195 °C; $[\alpha]_D^{22}$ –4.29 (c 0.14, MeOH); ν_{max}/cm^{-1} (ATR) 3291, 2918, 2850, 1630, 1563, 1550, 1526, 1500, 1472, 1435, 1398, 1375, 1360, 1295, 1273, 1222, 1188, 1133, 1106,

1012, 912, 857, 823, 800, 784, 751, 722, 669, 648, 627; δ_H (500 MHz; DMSO-d_6; Me_4Si) 0.85 (t, J = 6.8 Hz, 3H), 1.07–1.42 (m, 24H), 1.54 (p, J = 7.1 Hz, 2H), 1.70 (d, J = 6.9 Hz, 3H), 3.36 (q, J = 6.5 Hz, 2H), 5.08–5.30 (m, 1H), 7.56 (d, J = 4.5 Hz, 1H), 7.98 (t, J = 5.8 Hz, 1H), 8.68 (s, 1H), 8.92 (d, J = 4.5 Hz, 1H), 9.11 (d, J = 5.0 Hz, 3H); δ_C (126 MHz; DMSO-d_6; Me_4Si) 14.4, 17.1, 22.6, 26.9, 29.2, 29.2, 29.4, 29.5, 29.5, 29.9, 31.8, 38.9, 45.3, 106.5, 107.3, 145.8, 146.0, 147.7, 152.5, 161.2; HRMS (ESI): MH^+, found 416.3388. $[C_{24}H_{42}N_5O]^+$ requires 416.3384.

*3.7. General procedure for the synthesis of compounds **15**.*

Compound **14** (0.5 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL). Diisopropylethylamine (DIPEA, 5 mmol) and acyl chloride (4 mmol) were added and the mixture was stirred at r.t. for 12 h. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with 1 M $NaHSO_4$ (2 × 15 mL), saturated $NaHCO_3$ (2 × 15 mL), and saturated NaCl solution (15 mL). The organic phase was dried over anhydrous Na_2SO_4 and volatile components were evaporated in vacuo. The residue was purified by CC (EtOAc/hexanes). Fractions containing the product were combined and evaporated in vacuo to give 15.

*3.7.1. Methyl (1'S)-7-[1-(acetamido)ethyl]pyrazolo[1,5-a]pyrimidine-3-carboxylate (**15a**).* Prepared from **14f** (87 mg, 0.34 mmol), DIPEA (592 µL, 3.4 mmol), and AcCl (193 µL, 2.7 mmol) following general procedure, CC was not applied. Pale brown solid (42 mg, 47%). Pale brown solid (42 mg, 47%); mp 154–161 °C; $[\alpha]_D^{22}$ –9.3 (c 0.20, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 2798, 2516, 2051, 1943, 1869, 1646, 1587, 1550, 1511, 1477, 1440, 1420, 1378, 1306, 1252, 1180, 1159, 1116, 1043, 1021, 940, 852, 821, 795, 758, 689, 643; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.71 (d, J = 7.2 Hz, 3H), 2.02 (s, 3H), 3.98 (s, 3H), 5.65 (dq, J = 8.7, 7.1 Hz, 1H), 6.97 (d, J = 4.3 Hz, 1H), 7.01 (d, J = 8.7 Hz, 1H), 8.62 (s, 1H), 8.74 (d, J = 4.3 Hz, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.5, 23.3, 46.8, 51.8, 103.0, 107.2, 147.5,

148.3, 149.3, 153.0, 162.9, 169.5; HRMS (ESI): MH^+, found 263.1138. $[C_{12}H_{15}N_4O_3]^+$ requires 263.1139.

*3.7.2. Methyl (1'S)-7-[1-(benzamido)ethyl]pyrazolo[1,5-a]pyrimidine-3-carboxylate (**15b**).* Prepared from **14f** (121.5 mg, 0.47 mmol), DIPEA (819 μL, 4.7 mmol), and PhCOCl (437 μL, 3.76 mmol) following general procedure, CC (EtOAc-hexanes, 1:1 and EtOAc-MeOH, 5:1). Pale brown solid (130 mg, 85%); mp 148–155 °C; $[\alpha]_D^{22}$ –1.24 (c 0.17, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3284, 1702, 1635, 1519, 1581, 1531, 1488, 1434, 1378, 1331, 1306, 1279, 1260, 1217, 1203, 1169, 1135, 1095, 1026, 1000, 981, 845, 915, 877, 843, 831, 802, 781, 759, 735, 697, 660, 628; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.83 (d, *J* = 7.2 Hz, 3H), 3.98 (s, 3H), 5.87 (dq, = *J* 8.7, 7.2 Hz, 1H), 7.05 (d, *J* = 4.4 Hz, 1H), 7.44 (dd, *J* = 8.3, 6.9 Hz, 2H), 7.49–7.56 (m, 1H), 7.80 (d, *J* = 7.0 Hz, 2H), 7.91 (d, *J* = 8.7 Hz, 1H), 8.67 (s, 1H), 8.76 (d, *J* = 4.3 Hz, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.7, 47.4, 51.8, 103.0, 107.4, 127.1, 128.7, 132.0, 133.5, 147.6, 148.4, 149.3, 153.1, 162.9, 166.6; HRMS (ESI): MH^+, found 325.1292. $[C_{17}H_{17}N_4O_3]^+$ requires 325.1295.

*3.7.3. Methyl (1'S)-7-[1-(phenylacetamido)ethyl]pyrazolo[1,5-a]pyrimidine-3-carboxylate (**15c**).* Prepared from **14f** (120 mg, 0.47 mmol), DIPEA (819 μL, 4.7 mmol), and BnCOCl (497 μL, 3.76 mmol) following general procedure, CC (EtOAc-hexanes, 2:1 and 1:0). White solid (108 mg, 68%); mp 167–173 °C; $[\alpha]_D^{22}$ –1.4 (c 0.16, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3279, 1707, 1645, 1619, 1547, 1488, 1435, 1377, 1354, 1260, 1215, 1202, 1169, 1144, 1096, 1031, 982, 963, 915, 842, 802, 781, 757, 734, 962, 661, 627, 610; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.62 (d, *J* = 7.2 Hz, 3H), 3.58 (d, *J* = 4.8 Hz, 2H), 3.98 (s, 3H), 5.61 (dq, *J* = 8.7, 7.1 Hz, 1H), 6.86 (d, *J* = 4.3 Hz, 1H), 7.00 (d, *J* = 8.7 Hz, 1H), 7.19 (dd, *J* = 7.6, 1.9 Hz, 2H), 7.30–7.37 (m, 3H), 8.45 (s, 1H), 8.70 (d, *J* = 4.3 Hz, 1H); δ_C (126 MHz;

$CDCl_3$; Me_4Si) 18.5, 43.7, 46.9, 51.8, 102.9, 107.0, 127.5, 129.1, 129.4, 134.2, 147.2, 148.2, 149.2, 152.9, 162.9, 170.6; HRMS (ESI): MH^+, found 339.1452. $[C_{18}H_{19}N_4O_3]^+$ requires 339.1452.

*3.7.4. (1'S)-7-[1-(Phenylacetamido)ethyl]pyrazolo[1,5-a]pyrimidine-3-(N-methylcarboxamide) (**15d**).* Prepared from **14h** (40 mg, 0.18 mmol), DIPEA (313 μL, 1.8 mmol), and BnCOCl (190 μL, 1.44 mmol) following general procedure, CC was not applied. Pale yellow solid (45 mg, 85%); mp 153–160 °C; $[\alpha]_D^{22}$ –8.5 (c 0.16, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3286, 3029, 1698, 1638, 1613, 1561, 1531, 1496, 1451, 1408, 1350, 1279, 1261, 1243, 1198, 1166, 1073, 1028, 959, 943, 829, 812, 777, 752, 699, 632 cm-1; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.62 (d, J = 7.2 Hz, 3H), 3.06 (d, J = 4.9 Hz, 3H), 3.57 (d, J = 7.3 Hz, 1H), 3.66 (s, 2H), 5.59 (dq, J = 8.9, 7.2 Hz, 1H), 6.83 (d, J = 4.3 Hz, 1H), 7.18–7.36 (m, 5H), 7.92 (q, J = 4.9 Hz, 1H), 8.51 (d, J = 4.3 Hz, 1H), 8.53 (s, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 18.4, 26.0, 41.0, 43.7, 47.1, 106.5, 127.6, 129.1, 129.4, 134.0, 146.0, 146.5, 149.1, 151.0, 162.8, 170.9; HRMS (ESI): MH^+, found 338.1614. $[C_{18}H_{20}N_5O_2]^+$ requires 338.1612.

*3.8. Synthesis of (1S)-N,N,N-Trimethyl-1-[3-(methoxycarbonyl)pyrazolo[1,5-a]pyrimidin-7-yl]ethan-1-ammonium iodide (**16a**).*

MeI (300 μL, 4.8 mmol) was added to a stirred suspension of **14f** (120 mg, 0.47 mmol) and K_2CO_3 (300 mg, 2.2 mmol) in MeOH (3 mL) and the mixture was stirred at r.t. for 3 days. The mixture was filtered and the solvent was evaporated in vacuo. Then, CH_2Cl_2 (50 mL) was added, the mixture was stirred for 30 min, and filtered. The filtrate was evaporated in vacuo to give **16a**. Pale brown solid (110 mg, 60%); mp 114–118 °C (decomp.); $[\alpha]_D^{22}$ –3.65 (c 0.17, MeOH); ν_{max}/cm^{-1} (ATR) 3004, 1698, 1618, 1551, 1481, 1437, 1388, 1356, 1279, 1254, 1220, 1167, 1103, 1068, 1014, 964, 948, 959, 832, 802, 782, 759, 661, 636; δ_H (500 MHz; DMSO-d_6; Me_4Si) 1.87 (d, J = 7.1 Hz, 3H), 3.18 (s, 9H), 3.85

(s, 3H), 5.93 (q, *J* = 7.1 Hz, 1H), 7.80 (d, *J* = 4.5 Hz, 1H), 8.77 (s, 1H), 8.97 (d, *J* = 4.4 Hz, 1H); δ_C (126 MHz; DMSO-d_6; Me_4Si) 14.7, 51.8, 51.9, 65.1, 103.4, 112.9, 142.6, 146.9, 148.2, 153.5, 162.5; HRMS (ESI): MH^+, found 263.1505. $[C_{13}H_{19}N_4O_2]^+$ requires 263.1503; (Found: C, 40.01; H, 4.95; N, 14.20. $C_{13}H_{19}IN_4O_2$ requires C, 40.01; H, 4.91; N, 14.36%).

*3.9. Synthesis of N,N,N-Trimethyl-(1S)-1-[3-(pentadecylcarbamoyl)pyrazolo[1,5-a]pyrimidin-7-yl]ethan-1-ammonium iodide (**16b**).*

MeI (100 μL, 1.6 mmol) was added to a stirred suspension of **14j** (36 mg, 0.08 mmol) and K_2CO_3 (30 mg, 0.22 mmol) in MeOH (2 mL) and the mixture was stirred at r.t. for 3 days. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with 10% aq. $Na_2S_2O_3$ (5 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and the filtrate was evaporated in vacuo. The residue was purified by DVFC over silica gel (EtOAc and saturated KNO_3 in MeOH). Fractions containing the product were combined and evaporated in vacuo. The residue was stirred in CH_2Cl_2 (30 mL) at r.t. for 30 min, and filtered. The filtrate was evaporated in vacuo to give **16b**. White solid (22 mg, 60%); mp 137–142 °C; $[\alpha]_D22$ –16.8 (c 0.05, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3364, 2917, 2847, 1644, 1555, 1523, 1467, 1332, 1254, 1224, 1167, 1128, 1096, 1045, 961, 907, 868, 828, 778, 722, 641; δ_H (500 MHz; DMSO-d_6; Me_4Si) 0.82–0.88 (m, 3H), 1.17–1.37 (m, 24H), 1.54 (p, *J* = 7.0 Hz, 2H), 1.85 (d, *J* = 7.0 Hz, 3H), 3.16 (s, 9H), 3.27–3.45 (m, 2H), 5.92 (q, *J* = 7.1 Hz, 1H), 7.73 (d, *J* = 4.5 Hz, 1H), 8.02 (t, *J* = 5.8 Hz 1H), 8.68 (s, 1H), 8.92 (d, *J* = 4.4 Hz, 1H); δ_C (126 MHz; DMSO-d_6; Me_4Si) 14.4, 14.6, 22.6, 26.9, 29.2. 29.2, 29.5, 29.5, 30.0, 31.8, 38.8, 51.8, 65.1, 107.0, 112.2, 142.8, 145.5, 146.4, 152.3, 161.2; HRMS (ESI): MH+, found 458.3853. $[C_{27}H_{48}N_5O]^+$ requires 458.3853.

*3.10. General procedure for the synthesis of compounds **17**.*

Compound **10**, **11**, or **14** (0.5 mmol) was dissolved in MeOH (20 mL), under Ar 10% palladium on carbon (53 mg) was added, and the mixture was shaken at r.t. under 3.5 bar hydrogen for 4 h. The mixture was filtered through a short pad of Celite®, the filtrate was evaporated in vacuo, and the residue was purified by CC (EtOAc/hexanes). Fractions containing the product were combined and evaporated in vacuo to give the major isomer **17**.

*3.10.1 Methyl (7RS)-7-{[(tert-butoxy)carbonyl]amino}methyl]-4H,5H,6H,7H-pyrazolo[1,5-a]pyrimidine-3-carboxylate (**17a**).* Prepared from **10b** (132 mg, 0.43 mmol), CC was not applied. White solid (129 mg, 96%); mp 140–143 °C; $[\alpha]_D^{22}$ –14.0 (c 0.18, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3387, 3252, 1708, 1673, 1612, 1556, 1538, 1463, 1444, 1403, 1388, 1363, 1327, 1314, 1275, 1254, 1225, 1207, 1195, 1169, 1148, 1111, 1065, 993, 966, 935, 915, 873, 806, 779, 758, 695, 633, 615; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.44 (s, 9H), 1.99–2.08 (m, 1H), 2.11–2.20 (m, 1H), 3.36–3.55 (m, 2H), 3.70–3.87 (m, 5H), 4.16–4.25 (m, 1H), 5.47 (s, 1H), 5.86 (s, 1H), 7.61 (s, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 24.7, 28.4, 37.2, 43.7, 50.8, 55.2, 79.6, 93.8, 139.2, 148.5, 156.2, 164.5; HRMS (ESI): MH^+, found 311.1710. $[C_{14}H_{23}N_4O_4]^+$ requires 211.1714; (Found: C, 53.67; H, 7.24; N, 17.75. $C_{14}H_{22}N_4O_4 \cdot ¼H_2O$ requires C, 53.41; H, 7.20; N, 17.79%).

*3.10.2. Methyl (7R,1'S)-7-[(1-{[(tert-butoxy)carbonyl]amino}ethyl]-4H,5H,6H,7H-pyrazolo[1,5-a]pyrimidine-3-carboxylate (**17b**).* Prepared from **10i** (320 mg, 1.0 mmol), CC (EtOAc-hexanes, 2:1). White solid (230 mg, 71%). Physical and spectral data were in agreement with the literature data [28].

*3.10.3. (7R,1'S)-7-[(1S)-1-{[(tert-Butoxy)carbonyl]amino}ethyl]-4H,5H,6H,7H-pyrazolo[1,5-a]pyrimidine-3-carboxylic acid (**17c**).* Prepared from **11i** (73 mg, 0.24 mmol), CC (EtOAc-hexanes, 4:1). Colourless oil (69 mg, 93%); $[\alpha]_D^{22}$ –5.0 (c 0.17, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 2977, 1659, 1600, 1539, 1505, 1452, 1391, 1365, 1291, 1235, 1208, 1162, 1085, 1054, 993, 942, 920, 846, 783, 730, 646; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.08 (d, *J* = 6.7 Hz, 3H), 1.46 (s, 9H), 1.91–2.21 (m, 2H), 3.34–3.47 (m, 1H), 3.47–3.55 (m, 1H), 3.98–4.09 (m, 1H), 4.22 (p, *J* = 3.9 Hz, 1H), 5.86 (s, 1H), 6.06 (d, *J* = 9.7 Hz, 1H), 7.64 (s, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 15.7, 25.1, 28.4, 37.6, 49.4, 58.9, 79.5, 93.1, 139.8, 149.7, 155.5, 169.1; HRMS (ESI): MH^+, found 311.1718. $[C_{14}H_{23}N_4O_4]^+$ requires 311.1714.

*3.10.4. (7R,1'S)-7-[(1-{[(tert-Butoxy)carbonyl]amino}ethyl]-4H,5H,6H,7H-pyrazolo[1,5-a]pyrimidine-3-(N-methylcarboxamide (**17d**).* Prepared from **13b** (319 mg, 1.0 mmol), CC (EtOAc). White solid (160 mg, 50%); mp 187–189 °C; $[\alpha]_D^{22}$ –8.4 (c 0.15, CH_2Cl_2); ν_{max}/cm^{-1} (ATR) 3377, 3295, 2981, 1705, 1621, 1588, 1541, 1450, 1407, 1391, 1372, 1347, 1294, 1268, 1236, 1164, 1111, 1062, 1009, 986, 947, 921, 879, 846, 816, 778, 758, 722, 691, 650, 630, 609; δ_H (500 MHz; $CDCl_3$; Me_4Si) 1.04 (d, *J* = 6.4 Hz, 3H), 1.46 (s, 9H), 1.97–2.07 (m, 1H), 2.13 (dt, *J* = 14.0, 4.1 Hz, 1H), 2.91 (d, *J* = 4.7 Hz, 3H), 3.30–3.38 (m, 1H), 3.45 (dt, *J* = 10.5, 4.7 Hz, 1H), 4.04 (s, 1H), 4.21 (s, 1H), 5.62 (d, *J* = 5.6 Hz, 1H), 6.17 (d, *J* = 9.4 Hz, 1H), 6.28 (s, 1H), 7.39 (s, 1H); δ_C (126 MHz; $CDCl_3$; Me_4Si) 15.5, 25.6, 25.7, 28.5, 37.6, 49.5, 58.9, 79.3, 95.8, 135.4, 149.1, 155.4, 165.2; HRMS (ESI): MH^+, found 324.2029. $[C_{15}H_{26}N_5O_3]^+$ requires 324.2030; (Found: C, 55.40; H, 7.86; N, 21.21. $C_{15}H_{25}N_5O_3 \cdot {}^1\!/_8H_2O$ requires C, 55.32; H, 7.82; N, 21.51%).

Funding: This research was funded by Slovenian Research Agency (ARRS), research core funding no. P1-0179 and P1-0140.

Acknowledgments: We thank to EN-FIST Centre of Excellence (Ljubljana, Slovenia) for using the SuperNova diffractometer.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A. Supplementary material: ^{1}H and ^{13}C NMR spectra of new compounds.

References

[1] (a) Joule, J. A.; Mills, K. *Heterocyclic Chemistry*, 5th ed.; Wiley-Blackwell: New York, 2010; pp. 1–718, ISBN: 978-1-405-13300-5. (b) Patrick, G.L. *An Introduction to Medicinal Chemistry*, 5th ed.; Oxford University Press: Oxford, 2013, pp. 1–789, ISBN: 978-0-19-969739-7. (c) Pernerstorfer, J. Molecular Design and Combinatorial Compound Libraries. In *Handbook of Combinatorial Chemistry. Drugs, Catalysts, Materials*, Nicolaou, K. C., Hanko, R., Hartwig, W., Eds.; Wiley-VCH: Weinheim, 2002, Volume 2, pp. 725–742, ISBN: 0-471-49726-6. (d) Dolle, R. E. Solid-phase Synthesis of Heterocyclic Systems (Heterocycles Containing One Heteroatom). In *Handbook of Combinatorial Chemistry. Drugs, Catalysts, Materials*, Nicolaou, K. C., Hanko, R., Hartwig, W., Eds.; Wiley-VCH: Weinheim, 2002, Volume 2, pp. 643–684, ISBN: 0-471-49726-6.

[2] (a) Vagner, J.; Qu, H.; Hruby, V. J. Peptidomimetics, a synthetic tool of drug discovery. *Curr. Opin. Chem. Biol.* **2008**, *12*, 292–296, DOI: 10.1016/j.cbpa.2008.03.009. (b) Robinson, J. A.; DeMarco, S.; Gombert, F.; Moehle, K.; Obrecht, D. The design, structures and therapeutic potential of protein epitope mimetics. *Drug Discovery Today* **2008**, *13*, 944–951, DOI:

10.1016/j.drudis.2008.07.008. (c) Robinson, J. A. β-Hairpin Peptidomimetics: Design, Structures and Biological Activities. *Acc. Chem. Res.* **2008**, *41*, 1278–1288, DOI: 10.1021/ar700259k. (d) Hanessian, S.; Auzzas, L. The Practice of Ring Constraint in Peptidomimetics Using Bicyclic and Polycyclic Amino Acids. *Acc. Chem. Res.* **2008**, *41*, 1241–1251, DOI: 10.1021/ar8000052. (e) Wells, J. A.; McClendon, C. L. Reaching for high-hanging fruit in drug discovery at protein–protein interfaces. *Nature* **2007**, *450*, 1001–, DOI: 10.1038/nature06526.

[3] (a) Hanessian, S.; McNaughton-Smith, G.; Lombart, H.-G.; Lubell, W. D. Design and synthesis of conformationally constrained amino acids as versatile scaffolds and peptide mimetics. *Tetrahedron* **1997**, *53*, 12789–12854, DOI: 10.1016/S0040-4020(97)00476-6. (b) Halab, L.; Gosselin, F.; Lubell, W. D. Design, synthesis, and conformational analysis of azacycloalkane amino acids as conformationally constrained probes for mimicry of peptide secondary structures. *Biopolymers* **2000**, *55*, 101–122, DOI: 10.1002/1097-0282(2000)55:2<101::AID-BIP20>3.0.CO;2-O. (c) Cluzeau, J.; Lubell, W. D. Design, synthesis, and application of azabicyclo[X.Y.0]alkanone amino acids as constrained dipeptide surrogates and peptide mimics. *Biopolymers* **2005**, *80*, 98–150, DOI: 10.1002/bip.20213.

[4] (a) Regan, A. C. Pyrazolo[1,5-*c*]pyrimidine (73). In *Comprehensive heterocyclic chemistry III,* Katritzky, A. R., Ramsden, C. A., Scriven, E. F. V., Taylor, R. J. K., Eds.; Elsevier Science Ltd.: Oxford, UK, 2008, Volume 11, J. Cossy, Ed., pp. 577–577, ISBN: 978-0-08-045003-2; and references cited therein; (b) Elnagdi, M. H.; Elmoghayar, M. R. H.; Elgemeie, G. E. H. Chemistry of Pyrazolopyrimidines. *Adv. Heterocycl. Chem.* **1987**, *41*, 319–376, DOI: 10.1016/S0065-2725(08)60164-6.

[5] SciFinder Scholar Substructure search performed on November 2, 2018.

[6] Hwang, J. Y.; Windisch, M. P.; Jo, S.; Kim, H. C.; Kim, S.; Kim, H.; Lee, M. E.; Park, D.-S.; Park, E.; Ahn, S.; Cechetto, J.; Kim, J.; Liuzzi, M.; No, M. Z.; Lee, J. Discovery and

characterization of a novel 7-aminopyrazolo[1,5-a]pyrimidine analog as a potent hepatitis C virus inhibitor. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7297–7301, DOI: 10.1016/j.bmcl.2012.10.123.

[7] Ivashchenko, A. V.; Golovina, E. S.; Kadieva, M. G.; Kysil, V. M.; Mitkin, O. D.; Okun, I. M. Antagonists of serotonin 5-HT_6 receptors. III. Pyridine-substituted 3-(phenylsulfonyl)pyrazolo[1,5-*a*]pyrimidines: synthesis and structure – activity relationship. *Pharm. Chem. J.* **2012**, **46**, 406–410, DOI: 10.1007/s11094-012-0810-4.

[8] (a) Hanan, E. J.; van Abbema, A.; Barrett, K.; Blair, W. S.; Blaney, J.; Chang, C.; Eigenbrot, C.; Flynn, S.; Gibbons, P.; Hurley, C. A.; Kenny, J. R.; Kulagowski, J.; Lee, L.; Magnuson, S. R.; Morris, C.; Murray, J.; Pastor, R. M.; Rawson, T.; Siu, M.; Ultsch, M.; Zhou, A.; Sampath, D.; Lyssikatos, J. P. Discovery of Potent and Selective Pyrazolopyrimidine Janus Kinase 2 Inhibitors. *J. Med. Chem.*, **2012**, *55*, 10090–10107, DOI: 10.1021/jm3012239; (b) Asano, T.; Yamazaki, H.; Kasahara, C.; Kubota, H.; Kontani, T.; Harayama, Y.; Ohno, K.; Mizuhara, H.; Yokomoto, M.; Misumi, K.; Kinoshita, T.; Ohta, M.; Takeuchi, M. Identification, Synthesis, and Biological Evaluation of 6-[(6*R*)-2-(4-Fluorophenyl)-6-(hydroxymethyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrimidin-3-yl]-2-(2-methylphenyl)pyridazin-3(2*H*)-one (AS1940477), a Potent p38 MAP Kinase Inhibitor. *J. Med. Chem.* **2012**, *55*, 7772–7785, DOI: 10.1021/jm3008008; (c) Kosugi, T.; Mitchell, D. R.; Fujino, A.; Imai, M.; Kambe, M.; Kobayashi, S.; Makino, H.; Matsueda, Y.; Oue, Y.; Komatsu, K.; Imaizumi, K.; Sakai, Y.; Sugiura, S.; Takenouchi, O.; Unoki, G.; Yamakoshi, Y.; Cunliffe, V.; Frearson, J.; Gordon, R.; Harris, C. J.; Kalloo-Hosein, H.; Le, J.; Patel, G.; Simpson, D. J.; Sherborne, B.; Thomas, P. S.; Suzuki, N.; Takimoto-Kamimura, M. Mitogen-Activated Protein Kinase-Activated Protein Kinase 2 (MAPKAP-K2) as an Antiinflammatory Target: Discovery and in Vivo Activity of Selective Pyrazolo[1,5-*a*]pyrimidine Inhibitors Using a Focused Library and Structure-Based Optimization Approach. *J. Med. Chem.* **2012**, *55*, 6700–6715, DOI: 10.1021/jm300411k.

[9] Xu, J.; Liu, H.; Li, G.; He, Y.; Ding, R.; Wang, X.; Feng, M.; Zhang, S.; Chen, Y.; Li, S.; Zhao, M.; Li, Y.; Qi, C. Synthesis and Biological Evaluation of 7-(2-Chlorophenylamino)-5-((2-[^{18}F]fluoro-ethyoxy)methyl)pyrazolo[1,5-*a*]pyrimidine-3-carbonitrile as PET Tumor Imaging Agent. *Z. Naturforsch.* **2012**, *67B*, 827–834, DOI: 10.5560/ZNB.2012-0047.

[10] Lopez, L. C.; Dos-Reis, S.; Espargaro, A.; Carrodeguas, J. A.; Maddelein, M.-L.; Ventura, S.; Sancho, J. Discovery of Novel Inhibitors of Amyloid β-Peptide 1–42 Aggregation. *J. Med. Chem.* **2012**, *55*, 9521–9530, DOI: 10.1021/jm301186p.

[11] Inaoka, T.; Bilbe, G.; Ishibashi, O.; Tezuka, K.; Kumegawa, M. Kokubo, T. Molecular Cloning of Human cDNA for Cathepsin K: Novel Cysteine Proteinase Predominantly Expressed in Bone. *Biochem. Biophys. Res. Commun.* **1995**, *206*, 89–96, DOI: 10.1006/bbrc.1995.1013.

[12] Dossetter, A. G.; Beeley, H.; Bowyer, J.; Cook, C. R.; Crawford, J. J.; Finlayson, J. E.; Heron, N. M.; Heyes, C.; Highton, A. J.; Hudson, J. A.; Jestel, A.; Kenny, P. W.; Krapp, S.; Martin, S.; MacFaul, P. A.; McGuire, T. M.; Gutierrez, P. M.; Morley, A. D.; Morris, J. J.; Page, K. M.; Ribeiro, L. R.; Sawney, H.; Steinbacher, S.; Smith; C.; Vickers, M. (1*R*,2*R*)-N-(1-Cyanocyclopropyl)-2-(6-methoxy-1,3,4,5-tetrahydropyrido[4,3-*b*]indole-2-carbonyl)cyclohexanecarboxamide (AZD4996): A Potent and Highly Selective Cathepsin K Inhibitor for the Treatment of Osteoarthritis. *J. Med. Chem.* **2012**, *55*, 6363–6374, DOI: 10.1021/jm3007257.

[13] Crawford, J. J.; Kenny, P. W.; Bowyer, J.; Cook, C. R.; Finlayson, J. E.; Heyes, C.; Highton, A. J.; Hudson, J. A.; Jestel, A.; Krapp, S.; Martin, S.; Macfaul, P. A.; McDermott, B. P.; McGuire, T. M.; Morley, A. D.; Morris, J. J.; Page, K. M.; Ribeiro, L. R.; Sawney, H.; Steinbacher, S.; Smith, C.; Dossetter, A. G. Pharmacokinetic Benefits of 3,4-Dimethoxy Substitution of a Phenyl Ring and Design of Isosteres Yielding Orally Available Cathepsin K Inhibitors. *J. Med. Chem.* **2012**, *55*, 8827–8837, DOI: 10.1021/jm301119s.

[14] Borišek, J.; Vizovišek, M.; Sosnowski, P.; Turk, B.; Turk, D.; Mohar, B.; Novič, M. Development of N-(Functionalized benzoyl)-homocycloleucyl-glycinonitriles as Potent Cathepsin K Inhibitors. *J. Med. Chem.* **2015**, *58*, 6928–6937, DOI: 10.1021/acs.jmedchem.5b00746.

[15] Lecaille, F.; Bromme, D.; Lalmanach, G. Biochemical properties and regulation of cathepsin K activity. *Biochimie* **2008**, *90*, 208–, DOI: 10.1016/j.biochi.2007.08.011.

[16] Garnero, P.; Borel, O.; Byrjalsen, I.; Ferreras, M.; Drake, F. H.; McQueney, M. S.; Foged, N. T.; Delmas, P. D.; Delaisse, J. M. The collagenolytic activity of cathepsin K is unique among mammalian proteinases. *J. Biol. Chem.* **1998**, *273*, 32347–32352.

[17] Kafienah, W.; Bromme, D.; Buttle, D. J.; Croucher, L. J.; Hollander, A. P. Human cathepsin K cleaves native type I and II collagens at the N-terminal end of the triple helix. *Biochem. J.* **1998**, *331*, 727–732.

[18] Boonen, S.; Rosenberg, E.; Claessens, F.; Vanderschueren, D.; Papapoulos, S. Inhibition of cathepsin K for treatment of osteoporosis. *Curr. Osteoporos. Rep.* **2012**, *10*, 73–79, DOI: 10.1007/s11914-011-0085-9.

[19] Brömme, D.; Lecaille, F. Cathepsin K inhibitors for osteoporosis and potential off-target effects. *Expert Opin. Investig. Drugs* **2009**, *18*, 585–600, DOI: 10.1517/13543780902832661.

[20] Wang, Y.; Li, R.; Zheng, Z.; Yia, H.; Li, Z. Identification of novel cathepsin K inhibitors using ligand-based virtual screening and structure-based docking. *RSC Adv.* **2016**, *6*, 82961, DOI: 10.1039/C6RA14251F.

[21] Gauthier, J. Y.; Chauret, N.; Cromlish, W.; Desmarais, S.; Duong, L. T.; Falgueyret, J. P.; Kimmel, D. B.; Lamontagne, S.; Léger, S.; LeRiche, T.; Li, C. S.; Massé, F.; McKay, D. J.; Nicoll-Griffith, D. A.; Oballa, R. M.; Palmer, J. T.; Percival, M. D.; Riendeau, D.; Robichaud, J.; Rodan, G. A.; Rodan, S. B.; Seto, C.; Thérien, M.; Truong, V.-L.; Venuti, M. C.; Wesolowski,

G.; Young, R. N.; Zamboni, R.; Black, W. C. The discovery of odanacatib (MK-0822), a selective inhibitor of cathepsin K. *Bioorg. Med. Chem. Lett.* **2008**, 18, 923–928, DOI: 10.1016/j.bmcl.2007.12.047.

[22] Bone, H.G.; Dempster, D.W.; Eisman, J.A.; Greenspan, S.L.; McClung, M.R.; Nakamura, T.; Papapoulos, S.; Shih, W. J.; Rybak-Feiglin, A.; Santora, A. C.; Verbruggen, N.; Leung, A. T.; Lombardi, A. Odanacatib for the treatment of postmenopausal osteoporosis: development history and design and participant characteristics of LOFT, the Long-Term Odanacatib Fracture Trial. *Osteoporos. Int.* **2015**; *26*, 699–712, DOI: 10.1007/s00198-014-2944-6.

[23] Lindström, E.; Rizoska, B.; Henderson, I.; Terelius, Y.; Jerling, M.; Edenius, C.; Grabowska, U. Nonclinical and clinical pharmacological characterization of the potent and selective cathepsin K inhibitor MIV-711. *J. Transl. Med.* **2018**, *16*:125, DOI: 10.1186/s12967-018-1497-4.

[24] Regan, A. C. Pyrazolo[1,5-*c*]pyrimidine (73). In *Comprehensive heterocyclic chemistry III,* Katritzky, A. R., Ramsden, C. A., Scriven, E. F. V., Taylor, R. J. K., Eds.; Elsevier Science Ltd.: Oxford, UK, 2008, Volume 11, Cossy, J., Ed.; pp. 577–577, ISBN: 978-0-08-045003-2; and references cited therein. (b) Hajos, G.; Riedl, Z. Aza analogues of pyrazolo[1,5-*a*]pyridines containing additional nitrogen atoms in the six-membered ring. In *Science of synthesis, Houben-Weyl: methods of molecular transformations*, Neier, R., Ed.; Thieme: Stuttgart, Germany, 2006, Volume 12, pp. 667–678, ISBN: 3-13-1426-41-1.

[25] Nolte, B.; Sucholeiki, I.; Feuerstein, T.; Gallagher, B. M., Jr.; Wu, X.; Steeneck, C.; Gege, C.; Deng, H.; Van Veldhuizen, J.; Taveras, A. Heterobicyclic matrix metalloprotease inhibitors, their preparation, pharmaceutical compositions, and use in therapy. WO2008063671; *Chem. Abstr.* **2008**, *149*, 10030.

[26] Ahmetaj, S.; Velikanje, N.; Grošelj, U.; Šterbal, I.; Prek, B.; Golobič, A.; Kočar, D.; Dahmann, G.; Stanovnik, B.; Svete, J. Parallel synthesis of 7-heteroaryl-pyrazolo[1,5-*a*]pyrimidine-3-carboxamides. *Mol. Divers.* **2013**, *17*, 731–743, DOI: 10.1007/s11030-013-9469-3.

[27] Drev, M.; Grošelj, U.; Mevec, Š.; Pušavec, E.; Štrekelj, J.; Golobič, A.; Dahmann, G.; Stanovnik, B. Svete, J. Regioselective synthesis of 1- and 4-substituted 7-oxopyrazolo[1,5-*a*]pyrimidine-3-carboxamides. *Tetrahedron* **2014**, *70*, 8267–8279, DOI: 10.1016/j.tet.2014.09.020.

[28] Šenica, L.; Petek, N.; Grošelj, U.; Svete, J. A Four-step Synthesis of Novel (*S*)-1-(heteroaryl)-1-aminoethanes from (*S*)-Boc-alanine. *Acta Chim. Slov.* **2015**, *62*, 60–71, DOI: 0.17344/acsi.2014.712.

[29] Lombar, K.; Grošelj, U.; Dahmann, G.; Stanovnik, B.; Svete, J. Synthesis of 6-Alkyl-7-oxo-4,5,6,7-tetrahydropyrazolo[1,5-*c*]pyrimidine-3-carboxamides. *Synthesis* **2015**, *47*, 497–506, DOI: 10.1055/s-0034-1379547.

[30] Lukić, B.; Grošelj, U.; Novinec, M.; Svete, J. Synthesis of Novel 5-(*N*-Boc-*N*-Benzyl-2-aminoethyl)-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidin-3carboxamides and Their Inhibition of Cathepsins B and K. *Acta Chim. Slov.* **2017**, *64*, 782–789, DOI: 10.17344/acsi.2017.3421.

[31] El Agamey, A. G. A.; El-Sakka, I.; El-Shahat, Z.; Elnagdi, M. H. Activated Nitriles in Heterocyclic Synthesis: Studies on the Chemistry of Antipyrin-4-ylacetonitrile. *Arch. Pharm.* **1984**, *317*, 289–293, DOI: 10.1002/ardp.19843170402.

[32] Ji, N.; Meredith, E.; Liu, D. L.; Adams, C. M.; Artman, G. D.; Jendza, K. C.; Ma, F. P.; Mainolfi, N.; Powers, J. J.; Zhang, C. Syntheses of 1-substituted-3-aminopyrazoles. *Tetrahedron Lett.* **2010**, *51*, 6799–6801, DOI: 10.1016/j.tetlet.2010.10.073.

[33] Li, J.; Schulte, M. L.; Nickels, M. L.; Manning, H. C. New structure–activity relationships of N-acetamide substituted pyrazolopyrimidines as pharmacological ligands of TSPO. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 3472–3477, DOI: 10.1016/j.bmcl.2016.06.041.

[34] Bera, H.; Ojha, P. K.; Tan, B. J.; Sun, L.; Dolzhenko, A. V.; Chui, W. K.; Chiu, G. N. C. Discovery of mixed type thymidine phosphorylase inhibitors endowed with antiangiogenic properties: Synthesis, pharmacological evaluation and molecular docking study of 2-thioxo-pyrazolo[1,5-*a*][1,3,5]triazin-4-ones. Part II. *Eur. J. Med. Chem.* **2014**, *78*, 294–303, DOI: 10.1016/j.ejmech.2014.03.063.

[35] Sanada, T.; Odaka, T.; Hasegawa, H.; Amano, H. Novel azomethine compound and thermal transfer sheet using the same azomethine compound pigment. WO 2010114038, *Chem. Abstr.* **2010**, *153*, 518143.

[36] Julian, P. L.; Oliver, J. J.; Kimball, R. H.; Pike, A. B.; Jefferson, G. D. α-Phenylacetonitrile. *Org. Synth.* **1938**, *18*, 66–68, DOI: 10.15227/orgsyn.018.0066.

[37] Martinelli, M. J.; Khau, V. V.; Horcher, L. M. A convenient synthesis of 4-ethyl-4-methyl-3-oxohexanenitrile via pinacol rearrangement. *J. Org. Chem.* **1993**, *58*, 5546–5547, DOI: 10.1021/jo00072a048.

[38] Searles, S.; Kash, H. M. The Structure of the so-called "6-Phenyl-1,4-dihydro-*as*-triazine". *J. Org. Chem.* **1954**, *19*, 928–929, DOI: 10.1021/jo01371a009.

[39] Lassagne, F.; Snegaroff, K.; Roisnel, T.; Nassar, E.; Mongin, F. Action of primary amines and hydroxylamine on ethoxymethyleneaminonaphtopyranes: synthesis of new naphtopyrano[2,3-*d*] pyrimidines derivatives. *Heterocycl. Commun.* **2005**, *11*, 139–144, DOI: 10.1515/HC.2005.11.2.139.

[40] Ege, G.; Gilbert, K.; Maurer, K. Reaktionen mit Diazoazolen, VIII[1)] Synthesen von Azolo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-onen. *Chem. Ber.* **1987**, *120*, 1375–1395, DOI: 10.1002/cber.19871200816.

[41] Farnum, D. G.; Yates, P. The Preparation and Reactions of Some Diazopyrazoles. *J. Am. Chem. Soc.* **1962**, *84*, 1399–1406, DOI: 10.1021/ja00867a013.

[42] Nenaidenko, V. G.; Golubinskii, I. V.; Lenkova, O. N.; Shastin, A. V.; Balenkova, E. S. New Synthesis of 3-Aryl-5-amino-1*H*-pyrazoles. *Russ. J. Org. Chem.* **2004**, *40*, 1518–1520, DOI: 10.1007/s11178-005-0053-x.

[43] Moe, S. T.; Thompson, A. B.; Smith, G. M.; Fredenburg, R. A.; Stein, R. L.; Jacobson, A. R. Botulinum neurotoxin serotype A inhibitors: Small-molecule mercaptoacetamide analogs. *Bioorg. Med. Chem.* **2009**, *17*, 3072–3079, DOI: 10.1016/j.bmc.2009.03.013.

[44] Nitz, T. J.; Salzwedel, K.; Finnegan, C.; Wild, C.; Brunton, S.; Flanagan, S.; Montalbetti, C.; Coulter, T. S.; Kimber, M.; Magaraci, F.; Johnston, D. Alpha-unsubstituted arylmethyl piperazine pyrazolo[1,5-*a*] pyrimidine amide derivatives. WO 2008134035, *Chem. Abstr.* **2008**, *149*, 534234.

[45] Šenica, L.; Grošelj, U.; Kasunič, M.; Kočar, D.; Stanovnik, B.; Svete, J. Synthesis of Enaminone-Based Vinylogous Peptides. *Eur. J. Org. Chem.* **2014**, 3067–3071, DOI: 0.1002/ejoc.201402033.

[46] Although formation of the minor 5-aminoalkyl substituted regioisomers was detected by TLC, these compounds were neither isolated nor characterized. Due to mild reaction conditions and because epimerisation has not been observed before in cyclisations of the non-racemic ynones **7**, even at elevated temperature [28, 45, and references cited therein], the chiral integrity of the products has not been controlled specifically.

[47] Downloaded from RSCB PDB Protein Data Bank, https://www.rcsb.org/structure/3KX1.

[48] Bossard, M. J.; Tomaszek, T. A.; Thompson, S. K.; Amegadzie, B. Y.; Hanning, C. R.; Jones, C.; Kurdyla, J. T.; McNulty, D. E.; Drake, F. H.; Gowen, M.; Levy, M. A. Proteolytic Activity of Human Osteoclast Cathepsin K. Expression, Purification, Activation, and Substrate Identification. *J. Biol. Chem.* **1996**, *271*, 12517–12524, DOI: 10.1074/jbc.271.21.12517.

[49] Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shaw, D. E.; Shelley, M.; Perry, J. K.; Francis, P.; Shenkin, P. S. Glide: A

New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking Accuracy. *J. Med. Chem.* **2004**, *47*, 1739–1749, DOI: 10.1021/jm0306430.

Hihglights of the manuscript:

Synthesis and biological evaluation of 7-(aminoalkyl)pyrazolo[1,5-*a*]pyrimidine derivatives as cathepsin K inhibitors.

Nejc Petek, Bogdan Štefane, Marko Novinec *, and Jurij Svete*

Highlights:

- ► Novel non-racemic pyrazolo[1,5-*a*]pyrimidine derivatives
- ► Simple synthetic method and broad scope
- ► Facile manipulation of functional groups and scaffold
- ► Structural diversity
- ► Inhibition of cathepsin K

Graphical Abstract

for the manuscript:

Synthesis and biological evaluation of 7-(aminoalkyl)pyrazolo[1,5-*a*]pyrimidine derivatives as cathepsin K inhibitors.

Nejc Petek, Bogdan Štefane, Marko Novinec *, and Jurij Svete*

University of Ljubljana, Faculty of Chemistry and Chemical Technology, Večna pot 113, 1000 Ljubljana, Slovenia.