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Development of novel PP2A activators for use in the treatment of acute myeloid leukaemia†

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AAL(S), the chiral deoxy analog of the FDA approved drug FTY720, has been shown to inhibit proliferation and apoptosis in several cancer cell lines. It has been suggested that it does this by activating protein phosphatase 2A (PP2A). Here we report the synthesis of new cytotoxic analogs of AAL(S) and the evaluation of their cytotoxicity in two myeloid cell lines, one of which is sensitive to PP2A activation. We show that these analogs activate PP2A in these cells supporting the suggested mechanism for their cytotoxic properties. Our findings identify key structural motifs required for anti-cancer effects.

Introduction

FTY720 (**1**) (fingolimod, Gilenya) is approved by the FDA as an immunosuppressant to treat multiple sclerosis.[1] The immunosuppressive properties of this molecule arise from *in vivo* phosphorylation of one of the alcohol groups, to afford the active metabolite, FTY720-P (**2**) (Fig. 1).[2] Interestingly, non-phosphorylated FTY720 (**1**) has been shown to induce apoptosis in a number of cancer cell lines.[3] While the exact mechanism of action is not known, apoptosis is achieved by indirectly activating protein phosphatase 2A (PP2A), which is an important phosphatase involved in growth factor signalling.[4] Inhibition of PP2A has been found to be crucial for the oncogenic effects of tyrosine kinases such as, BCR/ABL, c-KIT and FLT3-ITD[5] and has been shown to down regulate plasma membrane transporters of amino acids and glucose.[6] Importantly, FTY720 (**1**) is able to eliminate cancer cells which are resistant to current therapeutics without disruption to normal blood or bone marrow cells.[7]

Our lab has shown that tumorigenic, factor-independent FDC.P1 myeloid cells transduced with the mutant c-KIT oncogene (D816V), are more sensitive to PP2A activation than cells expressing wildtype c-KIT, or those transduced with an empty vector (EV), and that FTY720 (**1**) inhibits proliferation of the D816V cell line with 1.8 times selectivity, when compared to the EV cells.[8] However, the *in situ* phosphorylation of FTY720 limits FTY720's utility as a cancer drug. In contrast, AAL(S) (**3**), the chiral deoxy analog of FTY720 (**1**), has been shown to retain the PP2A activation and cytotoxic properties of FTY720 (**1**), but is not phosphorylated *in vivo* and as such, does not have the same immunosuppressive properties as FTY720 (**1**).[8] As a consequence, AAL(S) (**3**) has emerged as a molecule that could have value in the development of new cancer therapeutics. With this information, we sought to develop the cytotoxic properties of AAL(S) (**3**).

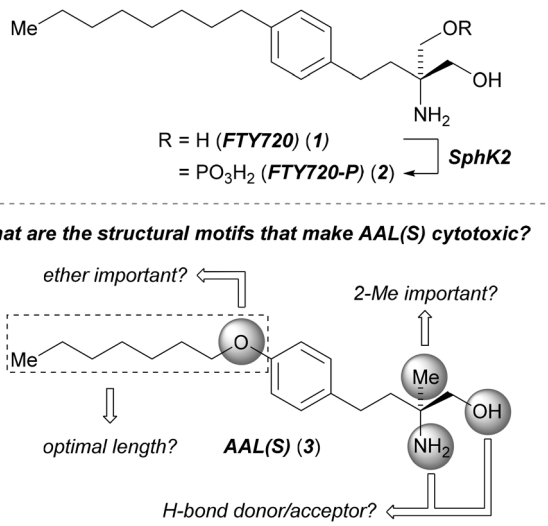

Fig. 1 Structures of FTY720 (**1**) and AAL(S) (**3**) and the regions of AAL(S) we chose to target as part of a structure–activity relationship study.

Recent work in our laboratories has focused on the development of an efficient synthetic strategy to access AAL(S) analogs and in a preliminary study we found that modifications to the hydrophobic tail moiety of AAL(S) (**3**) can have a major impact

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on the cytotoxicity in K562 leukaemia cells.[9] This paper extends this study and provides further insights into the role of each of the substituents of the quaternary aminoalcohol head group and the phenoxy ether group (Fig. 1) in an acute myeloid leukaemia model. A select number of the compounds were also tested for their ability to activate PP2A in cells to support the proposed mechanism of action.

Results and discussion

As a starting point in this investigation, we examined the ability of AAL(S) (**3**) to inhibit the growth of D816V and EV cells (Table 1). AAL(S) (**3**) behaves similarly to FTY720 (**1**), inducing cytotoxicity in D816V cells with an IC_{50} of 3.6 μM and 7.0 μM for the EV cells. This results in a slightly improved selectivity index of 1.9 compared to FTY720 (**1**). This confirms that the AAL(S) scaffold is an interesting starting point for developing FTY720 analogs that could be used in the treatment of leukaemia.

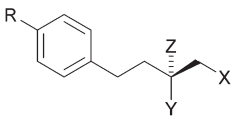
The hydrophobic tail analogs **4–11** have been previously synthesised by our group and our preliminary cytotoxicity results, using the K562 leukaemia cell line, indicated that the length of the chain can have profound effects on cytotoxicity.[9] Testing them in the FDC.P1-c-KIT-D816V cell line confirmed this trend. While activity is completely lost when the tail is shortened (<4 carbons long), is a benzyl group **10**, or a hydrophilic PEG-like tail **11**, the OC8 analog **6** was found to have a similar activity against D816V cells. However, there was deterioration of the selectivity for the D816V cells over the EV cells. Consequently, the OC7 tail was retained throughout the rest of our study.

Role of the 1-alcohol group (X)

As shown in Scheme 1, three analogs **12–14** (where X = H, F and OMe) were synthesised to investigate whether the 1-alcohol group had a role as either a hydrogen bond donor or acceptor, or whether it was required at all.

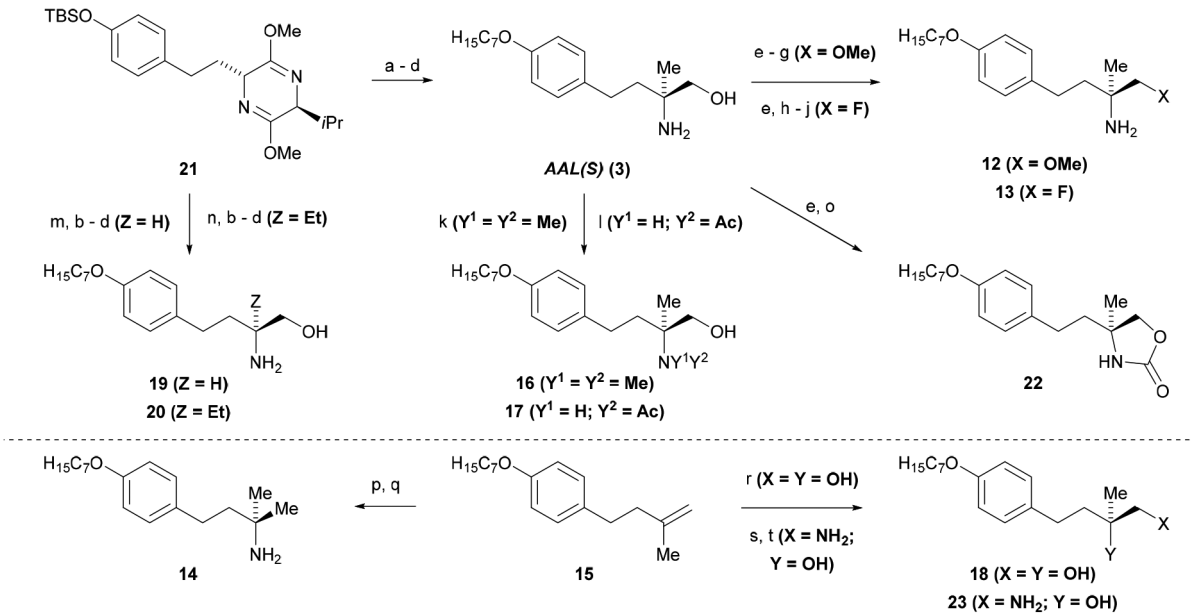
Both the 1-methoxy analog **12** and the 1-fluoro analog **13** could be generated from AAL(S) (**3**). The 1-methoxy analog **12** was synthesised in a 4 step sequence, as previously reported.[9] The synthesis of the 1-fluoro analog **13** began by firstly Boc-protection of the amino group of AAL(S) (**3**), using Boc_2O and saturated aqueous $NaHCO_3$ solution in EtOAc at reflux, to afford *N*-Boc AAL(S) in 83% yield. This material was converted into a cyclic sulfamidate, following the protocol developed by

Table 1 Cytotoxicity data for analogs of AAL(S) in FDC.P1 myeloid cells WT (EV) and mutant c-KIT$^+$ (D816V) cells and, for selected analogs, *in vivo* PP2A activation data



Compound number	R	X	Y	Z	IC_{50}[a] (μM) EV	IC_{50}[a] (μM) D816V	Selectivity	PP2A activation[b] (%)
FTY720 (**1**)	C_8H_{17}	OH	NH_2	CH_2OH	6.3	3.6	1.8[c]	150.1 ± 5.1
AAL(S) (**3**)	OC_7H_{15}	OH	NH_2	Me	7.0	3.7	1.9[c]	139.5 ± 6.7
4	OMe	OH	NH_2	Me	>9	>9	0	ND[d]
5	OC_4H_9	OH	NH_2	Me	>9	>9	0	ND
6	OC_8H_{17}	OH	NH_2	Me	5.0	3.6	1.4[c]	129.7 ± 1.5
7	OC_9H_{19}	OH	NH_2	Me	4.2	4.0	1.1	115.1 ± 6.4
8	$OC_{10}H_{21}$	OH	NH_2	Me	>9	>9	0	ND
9	$OC_{12}H_{25}$	OH	NH_2	Me	>9	>9	0	ND
10	OBn	OH	NH_2	Me	>9	>9	0	ND
11	$O(CH_2CH_2O)_2Me$	OH	NH_2	Me	>9	>9	0	ND
12	OC_7H_{15}	OMe	NH_2	Me	6.1	4.3	1.4[c]	136.8 ± 6.3
13	OC_7H_{15}	F	NH_2	Me	6.9	6.7	1.0	ND
14	OC_7H_{15}	H	NH_2	Me	4.0	3.1	1.3	127.5 ± 11.6
16	OC_7H_{15}	OH	NMe_2	Me	>9	7.5	≥1.2	118.5 ± 9.1
17	OC_7H_{15}	OH	NHAc	Me	>9	>9	0	ND
18	OC_7H_{15}	OH	OH	Me	>9	>9	0	ND
19	OC_7H_{15}	OH	NH_2	H	5.8	5.9	1.0	ND
20	OC_7H_{15}	OH	NH_2	Et	7.0	7.1	1.0	ND
22	OC_7H_{15}	–O(CO)NH–		Me	>9	>9	0	ND
23	OC_7H_{15}	NH_2	OH	Me	7.6	6.6	1.2	ND
24	OC_7H_{15}	OH	NH_2	CH_2OH	8.4	5.7	1.5[c]	131.7 ± 1.9
25	C_8H_{17}	OH	NH_2	Me	4.1	2.7	1.5[c]	137.0 ± 1.3

[a] Cells were treated with increasing concentrations of compound for 48 h. and viability determined using the resazurin assay.[5c] [b] 816 V myeloid cells were treated with 2.5 μM of compound for 12 h. PP2A activity was measured in PP2A immunoprecipitates by quantitation of phosphate release from a PP2A-specific phospho-peptide.[5c] Control was determined in untreated D816V cells as 100%. Data is mean ± SEM, $n = 3$. [c] $p < 0.01$ compared with EV, Student's *t* test. [d] ND: not determined.



Scheme 1 Reagents and yields (a) *n*-BuLi, THF, −78 °C, then MeI, −78 °C → 0 °C, 96%; (b) CsF, $H_{15}C_7Br$, DMF, rt, 72–88%; (c) TFA, H_2O, MeCN, rt, 25–99%; (d) $LiAlH_4$, THF, 0 °C → rt, 44–86%; (e) Boc_2O, sat. aq. $NaHCO_3$, EtOAc, Δ, 83%; (f) MeI, *n*-Bu_4NSO_4, 50% v/v 2 M aq. NaOH/THF, rt; (g) 2 M aq. HCl, MeCN, Δ, 82% (2 steps); (h) $SOCl_2$, py, MeCN, −40 °C → −10 °C, diastereomer 1 48%: diastereomer 2 46%; (i) $RuCl_3$, $NaIO_4$, 3 : 1 MeCN/H_2O, 0 °C, 85%; (j) *n*-Bu_4NF, MeCN, Δ, then 6 M aq HCl, Δ, 88%; (k) $NaCNBH_3$, $(CH_2O)_n$, AcOH, MeCN, 0 °C → rt, 54%; (l) AcCl, NEt_3, CH_2Cl_2, 0 °C → rt, 35%; (m) *n*-BuLi, THF, −78 °C, then MeOH, −78 °C, then 0 °C, 27% (59% **21**); (n) *n*-BuLi, THF, −78 °C, then EtI, −78 °C → 0 °C, 87%; (o) KOH, TsCl, Et_2O, Δ, 90%; (p) KCN, H_2SO_4, AcOH, 0 °C → rt, 97%; (q) 6 M aq. HCl, Δ, 96%; (r) $K_2OsO_4{\cdot}2H_2O$, K_2CO_3, $K_3Fe(CN)_6$, $(DHQD)_2PHAL$, 1 : 1 *t*-BuOH/H_2O, 0 °C → rt, 83%; (s) $TMSEOCONH_2$, $(DHQD)_2AQN$, $K_2OsO_4{\cdot}2H_2O$, 1,3-dichloro-5,5-dimethylhydantoin, NaOH, 1 : 1 *n*-PrOH/H_2O, 77%; (t) 2 M HCl in Et_2O, MeOH, rt, 45%.

Hinterding and co-workers for AAL(R),[10] by treatment of Boc-AAL(S) with $SOCl_2$ and py in MeCN. This resulted in a mixture of diastereomeric cyclic sulfimidates which were oxidised to the cyclic sulfamidate using $RuCl_3$ and $NaIO_4$ in 3 : 1 MeCN/H_2O in 80% yield over the two steps. Ring opening of this intermediate, using TBAF in MeCN at reflux, followed by *in situ* deprotection of the Boc group with 6 M aqueous HCl solution at reflux resulted in the formation of 1-fluoro analog **13** in 88% yield.

The achiral amine **14**, was generated from the readily available alkene **15**[11] by using the Ritter protocol (KCN in H_2SO_4, AcOH, 0 °C → rt followed by treatment with 6 M aqueous HCl solution at reflux) to provide **14** in 94% yield over two steps.[12]

As detailed in Table 1, the 1-fluorinated analog **13** lost activity and selectivity, while the 1-methoxy 12 analog lost some potency but retained the selectivity. This data provides some evidence that the 1-hydroxy group of the AAL(S) scaffold could be acting as a hydrogen bond acceptor. In contrast, the 1-des-hydroxy analog **14** displayed a significant improvement in cytotoxicity relative to AAL(S) (**3**) and FTY720 against the D816V cells, albeit, with a decrease in selectivity (1.3) when compared to the EV cells.

Role of the amino group (Y)

The amino group of AAL(S) (**3**) would be expected to be protonated at physiological pH and thus, may be involved in an electrostatic interaction with a protein target. It was decided to examine its role by either functionalising the amino group or by looking at a isosteric replacement. The two amino analogs (2-*N*,*N*-dimethylamine **16** and 2-*N*-acetyl **17**) have been previously synthesised, but their biological activity against leukaemia cell lines has not been examined.[9]

The isosteric oxygen analog **18** was synthesised, starting from the alkene **15** that was required in the synthesis of **14**, using a Sharpless dihydroxylation protocol[13] ($K_2OsO_4{\cdot}2H_2O$, K_2CO_3, $K_3Fe(CN)_6$ and $(DHQD)_2PHAL$ in 1 : 1 *t*-BuOH/H_2O at 0 °C → rt) to afford dihydroxy analog **18** in 83% yield.

Significantly, all the analogs varying the 2-amino group lost cytotoxicity in both the D816V and EV cell lines, suggesting that the amine could be involved in an electrostatic interaction and that the binding pocket must be small and cannot tolerate any steric bulk.

Significance of the 2-methyl group (Z)

The role of the 2-methyl group was investigated by preparing a 2-des-methyl analog **19** and 2-ethyl analog **20.** The 2-ethyl analog **20** was synthesised by treating **21** with *n*-BuLi in THF at −78 °C and quenching the resulting anion with ethyl iodide to afford the corresponding bis-lactim ether in 87% yield.[14] The synthesis was completed using our two-step one-pot deprotection/alkylation protocol to install the hydrophobic tail, hydrolysis and reduction of the Schöllkopf group in 13% yield over the three steps. In line with results reported by Hinterding,[15] hydrolysis of the Schöllkopf group for this substrate

proved troublesome due to increased bulk of the ethyl group around the quaternary stereocentre resulting in the isolation of a considerable amount of partially hydrolysed products.

The 2-des-methyl analog **19** was synthesised by treating bis-lactim ether **21** with *n*-BuLi in THF at −78 °C. The resulting anion was quenched with MeOH and quickly warmed to 0 °C to epimerise the stereocentre. This resulted in the formation of the desired material **19** in 27% yield and recovery of the starting material in 59% yield. While **19** should be accessed much more efficiently by using the alternative enantiomer of Schöllkopf's reagent this epimerisation protocol allowed ready access to complete the synthesis of the analog.

Thus, the synthesis of 2-des-methyl analog **21** was completed in 3 steps using the same two step one-pot deprotection/alkylation protocol to afford **21** in 31% overall yield.

These analogs were found to be less cytotoxic, and with no selectivity for the D816V cells, which suggests that the methyl group is the optimal size and/or is helping maintain a favourable conformation of the aminoalcohol head group (Table 1).

Other aminoalcohol head group analogs

To synthesise the 1-alcohol (X) analogs it was initially envisaged that the Boc-protected AAL(S) intermediate could be transformed into a cyclic aziridine which, when treated with nucleophiles, could ring-open to substitute this position. However, treating Boc-AAL(S) under standard conditions used to generate aziridines (KOH and TsCl in refluxing Et_2O) resulted only in the isolation of oxazolidinone **22** in 90% yield, which must arise through intramolecular attack of the hydroxyl group.

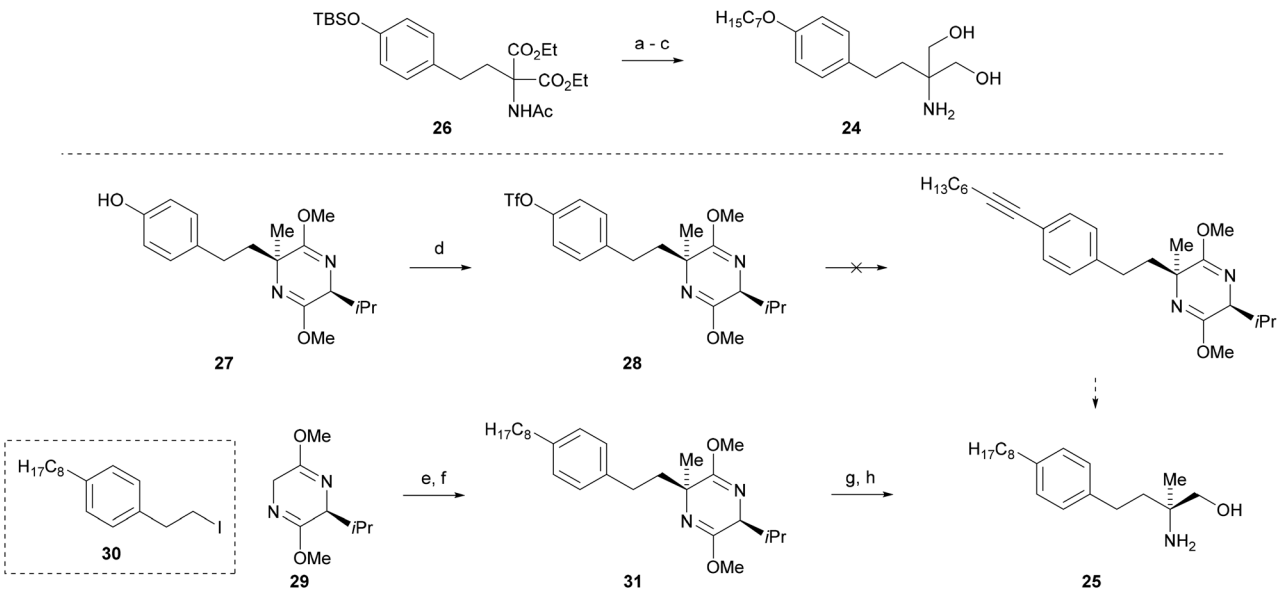
The AAL(S) regioisomer analog **23** was synthesised from alkene **15** using an aminohydroxylation reaction[16] (TMSEO-$CONH_2$, $(DHQD)_2AQN$, $K_2OsO_4{\cdot}2H_2O$, 1,3-dichloro-5,5-dimethyl-hydantoin and NaOH in 1 : 1 *n*-PrOH/H_2O) followed by TMSE deprotection with 2 M ethereal HCl in MeOH to afford **23** in 35% yield over 2 steps.

The cyclic oxazolidinone **22** and AAL(S) regioisomer **23** analogs also displayed poor activity in both D816V and the EV cell lines, which strengthens our hypothesis that the 2-amino group is involved in an electrostatic interaction in the binding site.

Phenoxy group

As the FTY720 tail is a C8 hydrocarbon, two FTY720-AAL(S) hybrid molecules **24** and **25** were synthesised so we could examine the importance of the phenoxy ether group of AAL(S) (**3**) (Scheme 2). The previously reported[8b,17] O-FTY720 analog **24** was prepared starting from TBS-protected ether 26 and using our two-step one-pot deprotection/alkylation strategy to install the tail. The synthesis was completed in 58% yield over the three steps.

To synthesise carbo-AAL(S) **25**, the counterpart to O-FTY720 (**24**), it was decided to utilise the previously prepared phenol **27**.[9] Conversion of 27 to the triflate **28** was achieved using Tf_2O and pyridine in CH_2Cl_2 in 46% yield. Despite many attempts at conducting a Sonagashira reaction on this substrate with 1-octyne, none of the desired material could be generated. To overcome this, (*S*)-Schöllkopf reagent **29** was alkylated with readily available 1-(2-iodoethyl)-4-octylbenzene (**30**),[11] followed by reaction with methyl iodide to generate bis-lactim ether **31** in 43% yield over the two steps. The synthesis was completed by hydrolysis and reduction of the Schöllkopf group to afford carbo-AAL(S) **25** in 45% yield over the two steps, as shown in Scheme 2.



Scheme 2 Reagents and yields (a) CsF, $H_{15}C_7Br$, DMF, rt, 94%; (b) $LiAlH_4$, THF, 0 °C → rt; (c) conc. HCl, EtOH, Δ, 62% (2 steps); (d) Tf_2O, py, CH_2Cl_2 0 °C, 46%; (e) *n*-BuLi, THF, −78 °C, then **30**, −78 °C → 0 °C, 83%; (f) *n*-BuLi, THF, −78 °C, then MeI, −78 °C → 0 °C, 52%; (g) TFA, H_2O, MeCN, rt, 65%; (h) $LiAlH_4$, THF, 0 °C → rt, 69%.

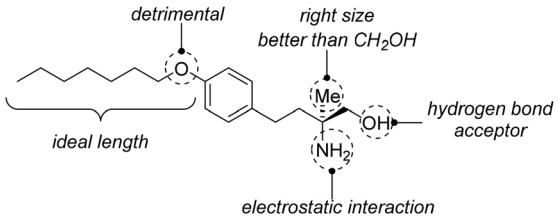


Fig. 2 SAR conclusions made from our study on the AAL(S) scaffold.

The biological data for these two analogs were found to be quite revealing, with O-FTY720 (**24**) having a much higher IC_{50} than AAL(S) (**3**) and FTY720 (**1**). In contrast, the carbo-AAL(S) analog 25 showed significantly improved cytotoxicity in comparison to both AAL(S) (**3**) and FTY720 (**1**), but importantly, maintained excellent selectivity for the D816V cells over the EV cells.

PP2A activation data

To further justify that the analogs that we had prepared were cytotoxic in our acute myeloid leukaemia model by activating PP2A, a select number of compounds were tested by treating D816V cells with 2.5 μM drug for 12 hours then immunopurifying PP2A complexes and measuring their phosphatase activity against a PP2A-specific phospho-peptide.[5c] Pleasingly, all of the compounds that we tested showed increased PP2A activity relative to the control, untreated cells (Table 1). Of particular interest was the result for the 1-des-hydroxy analog **14** as it had been hypothesised that the potent cytotoxicity may be a result of a mechanism not involving PP2A as the simplified structure is reminiscent of a detergent. However, this analog also showed increased PP2A activity, which supported our belief that these compounds operate by activating PP2A.

Conclusions

The synthesis of eight novel analogs of AAL(S) were developed so that an SAR investigation of the role of each of the substituents of the aminoalcohol head group as well as the phenoxy ether group in the hydrophobic tail of AAL(S) (**3**) could be carried out. These analogs, as well as fourteen previously reported analogs, were evaluated as cytotoxic agents using an acute myeloid leukaemia model which is sensitive to PP2A activation. The analogs showed varying cytotoxicity and revealed that some key structural motifs are critical to the cytotoxicity of the AAL(S) scaffold (Fig. 2). Importantly, it was shown that these analogs activate PP2A in oncogenic mutant c-KIT-D816V^{+} myeloid cells confirming the suggested mechanism of action for these compounds.

In particular, carbo-AAL(S) **25** had increased cytotoxicity in the D816V cell line albeit, with a small drop in selectivity over the non-tumorigenic control myeloid cell line. The cytotoxicity data obtained for O-FT720 (**24**) and carbo-AAL(S), and in comparison to AAL(S) (**3**) and FTY720 (**1**), suggests that the aminoalcohol head group of AAL(S) (**3**) is superior to that of FTY720 (**1**) in the design of PP2A activators. The structure–activity relationship data generated here will be valuable in our efforts to elucidate how these PP2A activators induce cell death in cancer cell lines.

Experimental

Chemistry[11]

Synthesis of (2*S*)-1-fluoro-4-(4′-heptyloxyphenyl)-2-methylbutan-2-amine (13). (a) Thionyl chloride (0.12 mL, 1.67 mmol) and pyridine (0.27 mL, 3.34 mmol) were added successively, dropwise to a solution of (2*S*)-*t*-butyl(1-hydroxy-4-(4′-heptyloxyphenyl)-2-methylbutan-2-yl)carbamate[9] (0.26 g, 0.67 mmol) in acetonitrile (15 mL) at −40 °C (dry ice/acetonitrile). The solution was allowed to warm slowly, in the cold bath, to −10 °C over 2.5 h. The reaction mixture was poured onto 1 M aqueous hydrochloric acid and extracted with ethyl acetate (×3). The organic extracts were combined and washed with saturate aqueous sodium bicarbonate, water and brine, the dried (Na_2SO_4). The solvent was removed under reduced pressure to afford (4*S*)-*t*-butyl-4-(4′-heptyloxyphenylethyl)-4-methyl-1,2,3-oxathiazolidine-3-carboxylate-2-oxide as a 1 : 1 mixture of diastereomers which were purified by flash chromatography on silica gel, eluting with 5% ethyl acetate/*n*-hexane, to afford one of the diastereomers as a clear colourless oil (0.14 g, 48%). $[\alpha]_D^{27.2}$ = −36 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.89 (t, J = 6.8 Hz, 3H), 1.26–1.47 (m, 8H), 1.45 (s, 3H), 1.54 (s, 9H), 1.71–1.81 (m, 2H), 2.00–2.10 (m, 1H), 2.28–2.39 (m, 1H), 2.44–2.55 (m, 1H), 2.59–2.69 (m, 1H), 3.92 (t, J = 6.6 Hz, 2H), 4.28 (d, J = 8.9 Hz, 1H), 4.96 (br d, J = 8.9 Hz, 1H), 6.79–6.84 (m, 2H), 7.04–7.10 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 14.2, 22.7, 26.2, 28.4, 29.2, 29.5, 30.2, 31.9, 40.0, 68.2, 83.8, 114.7, 129.3, 133.0, 157.7; IR (NaCl, neat) 1722 cm^{-1}; HRMS (ESI-MS): m/z calcd for $C_{23}H_{37}NO_5SNa$ $[M + Na]^+$ 462.2290, found 462.2277. Further elution afforded the other diastereomer as a clear, colourless oil (0.15 g, 51%). $[\alpha]_D^{27.2}$ = +40 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.89 (t, J = 6.8 Hz, 3H), 1.26–1.47 (m, 8H), 1.53 (s, 9H), 1.64 (s, 3H), 1.72–1.81 (m, 2H), 1.87 (td, J = 12.8, 5.2 Hz, 1H), 2.21 (td, J = 12.8, 4.6 Hz, 1H), 2.41 (td, J = 12.8, 4.8 Hz, 1H), 2.58 (td, J = 12.8, 5.2 Hz, 1H), 3.92 (t, J = 6.6 Hz, 2H), 4.59 (d, J = 9.2 Hz, 1H), 4.84 (br d, J = 9.2 Hz, 1H), 6.79–6.84 (m, 2H), 7.03–7.08 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 14.2, 22.7, 26.2, 28.4, 29.2, 29.4, 30.3, 31.9, 68.2, 83.9, 114.7, 129.2, 132.9, 157.7; IR (NaCl, neat) 1724 cm^{-1}; HRMS (ESI-MS): m/z calcd for $C_{23}H_{37}NO_5SNa$ $[M + Na]^+$ 462.2290, found 462.2279.

(b) Ruthenium(III) chloride (17 mg, 65 μmol) and sodium periodate (0.21 g, 0.99 mmol) were added successively to a solution of (4*S*)-*t*-butyl-4-(4′-heptyloxyphenylethyl)-4-methyl-1,2,3-oxathiazolidine-3-carboxylate-2-oxide (0.29 g, 0.66 mmol, 50 : 50 mixture of diastereomers) in 3 : 1 acetonitrile : water (40 mL) at 0 °C. The solution was stirred at 0 °C for 1 h then, the reaction solution was diluted with water and extracted with diethyl ether (×3). The organic extracts were combined and

washed with saturated aqueous sodium bicarbonate, water and brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crud material purified by flash chromatography on silica gel, eluting with 10% ethyl acetate/*n*-hexane, to afford (4*S*)-*t*-butyl-4-(4′-heptyloxyphenylethyl)-4-methyl-1,2,3-oxathiazolidine-3-carboxylate-2,2-dioxide as a clear colourless oil (0. 26 g, 85%). $[\alpha]_D^{27.2}$ = +22 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.89 (t, J = 6.8 Hz, 3H), 1.26–1.47 (m, 8H), 1.57 (s, 9H), 1.61 (s, 3H), 1.72–1.81 (m, 2H), 1.96 (m, 1H), 2.33–2.44 (m, 1H), 2.49–2.68 (m, 2H), 3.92 (t, J = 6.6 Hz, 2H), 4.16 (d, J = 9.2 Hz, 1H), 4.41 (d, J = 9.2 Hz, 1H), 6.80–6.85 (m, 2H), 7.04–7.09 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 14.2, 22.7, 23.4, 26.2, 28.1, 29.2, 29.37, 29.43, 31.9, 38.7, 65.0, 68.2, 73.9, 85.4, 114.8, 129.3, 132.2, 148.4, 157.9; IR (NaCl, neat) 1734 cm^{-1}; HRMS (ESI-MS): *m*/*z* calcd for $C_{23}H_{37}NO_6SNa$ $[M + Na]^+$ 478.2239, found 478.2228.

(c) A solution of tetra-*n*-butylammonium fluoride in THF (0.28 mL, 1.0 M, 0.28 mmol) was added dropwise to a solution of (4*S*)-*t*-butyl-4-(4′-heptyloxyphenylethyl)-4-methyl-1,2,3-oxathiazolidine-3-carboxylate-2,2-dioxide (51 mg, 0.11 mmol) in acetonitrile (3 mL) at room temperature. The solution was heated at reflux for 3 h. then, the solution was allowed to cool to room temperature and 6 M aqueous hydrochloric acid solution was added dropwise. The reaction mixture was heated at reflux for a further 3 h. after which, it was allowed to cool to room temperature. The acetonitrile was removed under reduced pressure. The residue was neutralised with solid sodium bicarbonate, diluted with water and extracted with ethyl acetate (×3). The organic extracts were combined and washed with water and brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with 1% triethylamine/ethyl acetate, to afford the title compound **13** as a clear colourless oil (29 mg, 88%) $[\alpha]_D^{26.2}$ = +4 (0.5, $CHCl_3$); ^{1}H NMR (600 MHz; $CDCl_3$) δ 0.89 (t, J = 6.8 Hz, 3H), 1.14 (d, J_{H-F} = 1.9 Hz, 3H), 1.28–1.47 (m, 10H), 1.67–1.73 (m, 2H), 1.74–1.79 (m, 2H), 2.56–2.66 (m, 2H), 3.92 (t, J = 6.6 Hz, 2H), 4.12 (dd, J = 17.7, 8.8 Hz, 1H), 4.20 (dd, J = 17.7, 8.8 Hz, 1H), 6.81–6.83 (m, 2H), 7.08–7.10 (m, 2H); ^{13}C NMR (150 MHz; $CDCl_3$) δ 14.2, 22.7, 23.8 (d, J_{C-F} = 4.2 Hz), 26.1, 29.2, 29.3, 29.4, 31.9, 41.2 (d, J_{C-F} = 2.9 Hz), 52.2 (d, J_{C-F} = 17.5 Hz), 68.2, 90.9 (d, J_{C-F} = 173.3 Hz), 114.6, 129.2, 134.1, 157.5; IR (NaCl, neat) 3294, 3370 cm^{-1}; HRMS (ESI-MS): *m*/*z* calcd for $C_{18}H_{31}FNO$ $[M + H]^+$ 296.2390, found 296.2384.

Synthesis of 1-(heptyloxy)-4-(3′-methylbut-3′-enyl)benzene (15). (a) 4-(4-Hydroxyphenyl)butan-2-one (1.33 g, 8.12 mmol) was added as a solid in one portion to a solution of cesium carbonate (2.91 g, 8.25 mmol) in dry DMF (12 mL) at room temperature. The solution was stirred for 15 min, then 1-bromoheptane (1.3 mL, 8.27 mmol) was added dropwise to the now bright yellow solution. The solution was stirred at room temperature for 24 h. The suspension was poured onto 2 M aqueous hydrochloric acid solution and extracted with ethyl acetate (×3). The organic extracts were combined and washed with water (×2) and brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with 10% ethyl acetate/*n*-hexane, to afford 4-(4′-(Heptyloxy)phenyl) butane-2-one as a clear colourless oil (1.94 g, 91%). ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.89 (t, J = 6.8 Hz, 3H), 1.31–1.49 (m, 8H), 1.72–1.81 (m, 2H), 2.12 (s, 3H), 2.69–2.74 (m, 2H), 2.81–2.86 (m, 2H), 3.92 (t, J = 6.6 Hz, 2H), 6.79–6.83 (m, 2H), 7.06–7.10 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 14.2, 22.7, 26.1, 29.0, 29.2, 29.4, 30.2, 31.9, 45.6, 68.1, 114.6, 129.3, 132.9, 157.7, 208.3; IR (NaCl, neat) 1717 cm^{-1}; HRMS (ESI-MS): *m*/*z* calcd for $C_{17}H_{26}NO_2Na$ $[M + Na]^+$ 285.1831, found 285.1827.

(b) A solution of *n*-butyllithium in hexanes (4.00 mL, 1.4 M, 5.40 mmol) was added dropwise to a solution of methyltriphenylphosphonium bromide (2.06 g, 5.76 mmol) in freshly distilled THF (50 mL) at room temperature. The solution was stirred at room temperature for 15 min where it had turned dark orange. A solution of 4-(4′-(Heptyloxy)phenyl)butane-2-one (1.01 g, 3.84 mmol) in freshly distilled THF (15 mL) was added dropwise after which, the reaction solution was stirred at room temperature for an additional 5 h. The reaction was quenched with water and the mixture extracted with *n*-pentane (×3). The organic extracts were combined and washed with water and brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with *n*-hexane, to afford the title compound **15** as a clear colourless oil (0.83 g, 83%). ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.90 (t, J = 6.8 Hz, 3H), 1.26–1.50 (m, 8H), 1.72–1.82 (m, 2H), 1.77 (s, 3H), 2.26–2.31 (m, 2H), 2.67–2.72 (m, 2H), 3.93 (t, J = 6.6 Hz, 2H), 4.70–4.71 (m, 1H), 4.73–4.74 (m, 1H), 6.79–6.84 (m, 2H), 7.07–7.12 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 14.2, 22.8, 26.2, 29.2, 29.5, 31.9, 33.5, 40.0, 68.2, 110.3, 114.5, 129.3, 134.2, 145.7, 157.4.

4-(4′-Heptyloxyphenyl)-2-methylbutan-2-amine (14). (a) Under a static atmosphere, a solution of concentrated sulfuric acid (0.2 mL, 1.11 mmol) in acetic acid (1 mL) was added dropwise to a solution of 1-(heptyloxy)-4-(3′-methylbut-3′-enyl) benzene[11] (0.19 g, 0.73 mmol) and potassium cyanide (48 mg, 0.74 mmol) in acetic acid (1 mL) at 0 °C. The solution was allowed to slowly warm to room temperature, in the cold bath, over 24 h. The reaction solution was basified to pH 12 with 2 M aqueous sodium hydroxide solution and extracted with diethyl ether (×3). The organic extracts were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with 50% ethyl acetate/*n*-hexane, to afford (2*S*)-*N*-(4-(4-heptyloxyphenyl)-1-hydroxy-2-methylbutan-2-yl)formamide as a 50 : 50 mixture rotamers, as a white solid (0.16 g, 97%). ^{1}H NMR (400 MHz; $CDCl_3$) δ 0.89 (t, J = 6.9 Hz, 3H, both rotamers), 1.28–1.46 (m, 20H), 1.36 (s, 6H, one rotamer), 1.40 (s, 6H, one rotamer), 1.72–1.82 (m, 8H, both rotamers), 1.99–2.03 (m, 2H, one rotamer), 2.52–2.60 (m, 4H), 3.91 (t, J = 6.6 Hz, 2H, one rotamer), 3.92 (t, J = 6.6 Hz, 2H, one rotamer), 5.25 (br s, 1H, one rotamer), 5.98 (br d, J = 12.0 Hz, one rotamer), 6.79–6.83 (m, 4H), 7.05–7.09 (m, 4H), 8.05 (d, J = 2.0 Hz, 1H, one rotamer), 8.28 (d, J = 12.0 Hz, 1H, one rotamer); ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.2, 22.7, 26.1, 27.3, 28.8, 29.18, 29.19, 29.43, 29.45, 29.51, 29.8, 31.9, 42.6, 46.1,

52.9, 54.1, 68.16, 68.17, 114.6, 114.7, 129.2, 129.3, 133.2, 133.9, 157.5, 157.7, 160.6, 163.2; IR (NaCl, neat) 1683, 3290 cm^{-1}; HRMS (ESI-MS): *m*/*z* calcd for $C_{19}H_{31}O_2Na$ $[M + Na]^+$ 328.2252, found 328.2247.

(b) (2*S*)-*N*-(4-(4-heptyloxyphenyl)-1-hydroxy-2-methylbutan-2-yl)formamide (56 mg, 0.18 mmol) in 6 M aqueous hydrochloric acid solution (9 mL) was heated at reflux for 16 h. The solution was cooled to room temperature and basified to pH 14 using 2 M aqueous sodium hydroxide solution. The suspension was extracted with dichloromethane (×3). The organic extracts were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure to afford the title compound **14** as a clear colourless oil (49 mg, 96%). ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.89 (t, J = 6.8 Hz, 3H), 1.16 (s, 6H), 1.29–1.46 (m, 10H), 1.61–1.67 (m, 2H), 1.71–1.81 (m, 2H) 2.56–2.61 (m, 2H), 3.92 (t, J = 6.6 Hz, 2H), 6.79–6.84 (m, 2H), 7.07–7.12 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 14.2, 22.7, 26.2, 29.2, 29.5, 30.3, 30.5, 31.9, 47.5, 49.6, 68.2, 114.6, 129.2, 134.7, 157.4; IR (NaCl, neat) 3189, 3284, 3353 cm^{-1}; HRMS (ESI-MS): *m*/*z* calcd for $C_{18}H_{32}NO$ $[M + H]^+$ 278.2484, found 278.2480.

(2*S*)-4-(4′-Heptyloxyphenyl)-2-methylbutane-1,2-diol (18). Potassium osmate dihydrate (1 mg, 2.71 μmol) was added as a solid in one portion to a suspension of 1-(heptyloxy)-4-(3′-methylbut-3′-enyl)benzene[11] (0.13 g, 0.48 mmol), potassium carbonate (0.20 g, 1.45 mmol), potassium ferricyanide (0.48 g, 1.45 mmol) and $(DHQD)_2PHAL$ (20 mg, 24 μmol) in 1 : 1 *t*-butanol : water (8 mL) at 0 °C. The solution was allowed to slowly warm to room temperature, in the cold bath, over 20 h. Sodium sulfite (0.55 g, 4.35 mmol) was added and the solution allowed to stir for a further 15 min. The mixture was diluted with water and extracted with ethyl acetate (×3). The organic extracts were combined and washed with brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with 60% ethyl acetate/*n*-hexane, to afford the title compound **18** as a clear colourless oil (0.12 g, 83%). $[\alpha]_D^{21.0}$ = −4 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.91 (t, J = 6.8 Hz, 3H), 1.22 (s, 3H), 1.32–1.48 (m, 8H), 1.73–1.82 (m, 4H), 2.58–2.67 (m, 2H), 2.94 (br s, 1H), 3.29 (br s, 1H), 3.42 (d, J = 11.0 Hz, 1H), 3.50 (d, J = 11.0 Hz, 1H), 3.91 (t, J = 6.6 Hz, 2H), 6.79–6.83 (m, 2H), 7.08–7.11 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 14.2, 22.7, 23.2, 26.1, 29.16, 29.21, 29.4, 31.9, 40.7, 68.1, 69.8, 73.1, 114.6, 129.2, 134.2, 157.4; IR (NaCl, neat) 3352 cm^{-1}; HRMS (ESI-MS): *m*/*z* calcd for $C_{18}H_{30}O_3Na$ $[M + Na]^+$ 317.2093, found 317.2093.

Synthesis of (2*S*)-2-amino-4-(4′-heptyloxyphenyl)butan-1-ol (19). (a) A solution of *n*-butyllithium in hexanes (0.17 mL, 2.1 M, 0.36 mmol) was added dropwise to a solution of (2*R*,5*S*)-5-isopropyl-3,6-dimethoxy-2-(4′-*t*-butyldimethylsilyloxyphenethyl)-2,5-dihydropyrazine[10] (0.15 g, 0.35 mmol) in freshly distilled THF (2 mL) at −78 °C (dry ice/acetone). The solution was stirred at −78 °C for 15 min after which, it had turned dark yellow. Methanol (28 μL, 0.69 mmol) was added dropwise and the solution allowed to warm slowly, in the cold bath, to −40 °C over 3 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution and allowed to warm to room temperature. The THF was removed under reduced pressure and the residue extracted with dichloromethane (×4). The organic extracts were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure to afford a yellow oil. ^{1}H NMR analysis showed the diastereometric ratio to be 70 : 30, in favour of the starting material, through integration of the benzylic multiplets at δ 2.46–2.62 and δ 2.65–2.81 respectively. The mixture was purified by flash chromatography on silica gel, eluting with 3% ethyl acetate/*n*-hexane, to afford the starting material as a clear colourless oil (89 mg, 59%). All spectroscopic data matched those reported previously.[10] Further elution afforded (2*S*,5*S*)-5-isopropyl-3,6-dimethoxy-2-(4′-*t*-butyldimethyl-silyloxyphenethyl)-2,5-dihydropyrazine as a clear colourless oil (41 mg, 27%). $[\alpha]_D^{25.0}$ = −6 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.18 (s, 6H), 0.75 (d, J = 6.8 Hz, 3H), 0.98 (s, 9H), 1.08 (d, J = 6.8 Hz, 3H), 1.68–1.80 (m, 1H), 2.12–2.29 (m, 2H), 2.65–2.81 (m, 2H), 3.68 (s, 3H), 3.71 (s, 3H), 3.94–3.03 (m, 2H), 6.73–6.78 (m, 2H), 7.05–7.10 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ −4.3, 17.7, 18.3, 19.7, 25.9, 31.4, 31.6, 37.6, 52.38, 52.41, 55.2, 61.1, 120.0, 129.5, 135.0, 153.8, 163.2, 163.9; IR (NaCl, neat) 1697 cm^{-1}; HRMS (ESI-MS): *m*/*z* calcd for $C_{23}H_{39}N_2O_3Si$ $[M + Na]^+$ 419.2729, found 419.2714.

(b) Cesium fluoride (0.20 g, 1.33 mmol) was added as a solid in one portion to a solution of (2*S*,5*S*)-5-isopropyl-3,6-dimethoxy-2-(4′-*t*-butyldimethylsilyloxyphenethyl)-2,5-dihydropyrazine (0.28 g, 0.67 mmol) in dry DMF (5 mL) at room temperature. The solution was stirred for 15 min, where it had turned dark orange, before 1-bromoheptane (0.20 mL, 1.27 mmol) was added dropwise. The solution was stirred at room temperature for 17 h. Water was added and the mixture was extracted with ethyl acetate (×3). The organic extracts were combined and washed with water and brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with 2% ethyl acetate/*n*-hexane, to afford (2*S*,5*S*)-5-isopropyl-3,6-dimethoxy-2-(4′-heptyloxyphenethyl)-2,5-dihydropyrazine as a clear colourless oil (0.19 g, 72%). $[\alpha]_D^{25.0}$ = +44 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.74 (d, J = 6.8 Hz, 3H), 0.89 (t, J = 6.8 Hz, 3H), 1.07 (d, J = 6.8 Hz, 3H), 1.25–1.49 (m, 8H), 1.70–1.81 (m, 3H), 2.12–2.29 (m, 2H), 2.66–2.81 (m, 2H), 3.68 (s, 3H), 3.71 (s, 3H), 3.92 (t, J = 6.6 Hz, 2H), 3.94–4.02 (m, 2H), 6.79–6.84 (m, 2H), 7.11–7.15 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 14.2, 17.7, 19.7, 22.8, 26.2, 29.2, 29.5, 31.4, 31.5, 31.9, 37.7, 52.39, 52.42, 55.2, 61.0, 68.2, 114.5, 129.5, 134.2, 157.5, 163.2, 163.9; IR (NaCl, neat) 1693 cm^{-1}; HRMS (ESI-MS): *m*/*z* calcd for $C_{24}H_{39}N_2O_3$ $[M + H]^+$ 403.2961, found 403.2959.

(c) A solution of TFA (2.5 mL) in water (5 mL) was added dropwise to a solution of (2*S*,5*S*)-5-isopropyl-3,6-dimethoxy-2-(4′-heptyloxyphenethyl)-2,5-dihydropyrazine (0.19 g, 0.48 mmol) in acetonitrile (12 mL). The solution was stirred at room temperature for 4 h after which the acetonitrile was removed under reduced pressure. The residue was diluted with water and neutralised with portions of solid sodium bicarbonate, then extracted with dichloromethane (×4). The organic extracts were combined and dried (Na_2SO_4). The solvent was

removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with ethyl acetate, to afford methyl (2*S*)-2-amino-4-(4′-heptyloxyphenyl) butanoate as a clear colourless oil (0.15 g, 99%). $[\alpha]_D^{22.3}$ = +14 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 3H), 1.26–1.46 (m, 8H), 1.53 (br s, 2H), 1.70–1.86 (m, 3H), 1.97–2.08 (m, 1H), 2.57–2.73 (m, 2H), 3.43 (dd, J = 7.9, 5.2 Hz, 1H) 3.69 (s, 3H), 3.91 (t, J = 6.6 Hz, 2H), 6.78–6.83 (m, 2H), 7.06–7.11 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 14.1, 22.7, 26.1, 29.1, 29.4, 31.1, 31.9, 36.7, 52.0, 53.9, 68.1, 114.5, 129.4, 133.1, 157.6, 176.6; IR (NaCl, neat) 1738, 3317, 3382 cm^{-1}; HRMS (ESI-MS): m/z calcd for $C_{18}H_{29}NO_3Na$ $[M + Na]^+$ 330.2045, found 330.2031.

(d) Lithium aluminium hydride (37 mg, 0.98 mmol) was added as a solid, in one portion, to a solution of methyl (2*S*)-2-amino-4-(4′-heptyloxyphenyl)butanoate (0.15 g, 0.48 mmol) in freshly distilled THF (4 mL) at 0 °C. The solution was stirred at 0 °C for 20 min then the cold bath was removed and the solution stirred at room temperature for 2 h. The reaction was quenched with saturated aqueous sodium sulfate solution and the mixture was extracted with ethyl acetate (×4). The organic extracts were combined and washed with saturated aqueous sodium bicarbonate solution, water and brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with 3% methanol/3% triethylamine/dichloromethane, to afford the title compound **19** as a clear gum (59 mg, 44%). $[\alpha]_D^{26.4}$ = −2 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.89 (t, J = 6.8 Hz, 3H), 1.26–1.46 (m, 8H), 1.51–1.63 (m, 1H), 1.69–1.81 (m, 3H), 2.07 (br s, 1H), 2.17 (br s, 2H), 2.54–2.73 (m, 2H), 2.83–2.91 (m, 1H), 3.32 (dd, J = 10.6, 7.8 Hz, 1H), 3.61 (dd, J = 10.6, 3.5 Hz, 1H), 3.91 (t, J = 6.6 Hz, 2H), 6.69–6.83 (m, 2H), 7.06–7.09 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 14.2, 22.8, 26.2, 29.2, 29.5, 31.6, 31.9, 36.3, 52.5, 66.6, 68.2, 114.7, 129.3, 133.6, 157.6; IR (NaCl, neat) 3120, 3280, 3339 cm^{-1}; HRMS (ESI-MS): m/z calcd for $C_{17}H_{30}NO_2$ $[M + H]^+$ 280.2277, found 280.2265.

Synthesis of (2*S*)-2-amino-2-ethyl-4-(4′-heptyloxyphenyl) butan-1-ol (20). (a) A solution of *n*-butyllithium in hexanes (0.47 mL, 2.1 M, 0.99 mmol) was added dropwise to a solution of (2*R*,5*S*)-5-isopropyl-3,6-dimethoxy-2-(4′-*t*-butyldimethylsilyloxyphenethyl)-2,5-dihydropyrazine[10] (0.39 g, 0.94 mmol) in freshly distilled THF (6 mL) at −78 °C (dry ice/acetone). The solution was stirred at −78 °C for 15 min after which, it had turned dark yellow. Ethyl iodide (83 μL, 1.03 mmol) was added dropwise and the solution allowed to warm slowly, in the cold bath, to −10 °C over 5 h. The reaction was quenched with saturated aqueous sodium bicarbonate solution and allowed to warm to room temperature. The solvent was removed under reduced pressure and the residue extracted with dichloromethane (×4). The organic extracts were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with 3% ethyl acetate/*n*-hexane, to afford (2*S*,5*S*)-5-isopropyl-3,6-dimethoxy-2-(4′-*t*-butyldimethylsilyloxyphenethyl)-2-ethyl-2,5-dihydropyrazine as a clear colourless oil (0.37 g, 87%). $[\alpha]_D^{24.3}$ = + 36 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.17 (s, 6H), 0.66 (t, J = 7.4 Hz, 3H), 0.71 (d, J = 6.8 Hz, 3H), 0.97 (s, 9H), 1.12 (d, J = 6.8 Hz, 3H), 1.51–1.63 (m, 1H), 1.75–1.90 (m, 2H), 2.09 (td, J = 12.8, 4.2 Hz, 1H), 2.21 (td, J = 12.8, 4.2 Hz, 1H), 2.32–2.46 (m, 2H), 3.71 (s, 3H), 3.72 (s, 3H), 3.93 (d, J = 3.3 Hz, 1H), 6.71–6.75 (m, 2H), 6.98–7.02 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ −4.3, 8.4, 17.2, 18.4, 19.7, 25.9, 30.78, 30.81, 34.0, 42.4, 52.3, 52.4, 60.9, 62.7, 119.9, 129.3, 135.5, 153.6, 162.9, 164.1; IR (NaCl, neat) 1691 cm^{-1}; HRMS (ESI-MS): m/z calcd for $C_{25}H_{42}N_2O_3SiNa$ $[M + Na]^+$ 469.2862, found 469.2845.

(b) Cesium fluoride (0.12 g, 0.77 mmol) was added as a solid in one portion to a solution of (2*S*,5*S*)-5-isopropyl-3,6-dimethoxy-2-(4′-*t*-butyldimethylsilyloxyphenethyl)-2-ethyl-2,5-dihydropyrazine (0.17 g, 0.39 mmol) in dry DMF (3.5 mL) at room temperature. The solution was stirred for 15 min, where it had turned dark orange, before 1-bromoheptane (90 μL, 0.44 mmol) was added dropwise. The solution was stirred at room temperature for 17 h. Water was added and the mixture was extracted with ethyl acetate (×3). The organic extracts were combined and washed with water and brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with 3% ethyl acetate/*n*-hexane, to afford (2*S*,5*S*)-5-isopropyl-3,6-dimethoxy-2-(4′-heptyloxyphenethyl)-2-ethyl-2,5-dihydropyrazine as a clear colourless oil (0.14 g, 84%). $[\alpha]_D^{24.8}$ = + 30 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.66 (t, J = 7.4 Hz, 3H), 0.71 (d, J = 6.8 Hz, 3H), 0.89 (t, J = 6.8 Hz, 3H), 1.12 (d, J = 6.8 Hz, 3H), 1.31–1.47 (m, 8H), 1.51–1.63 (m, 1H), 1.71–1.90 (m, 4H), 2.09 (td, J = 12.8, 4.2 Hz, 1H), 2.22 (td, J = 12.8, 4.2 Hz, 1H), 2.33–2.47 (m, 2H), 3.71 (s, 3H), 3.72 (s, 3H), 3.90–3.94 (m, 3H), 6.79–6.82 (m, 2H), 7.04–7.07 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 8.4, 14.2, 17.2, 19.7, 22.8, 26.2, 29.2, 29.5, 30.7, 30.8, 32.0, 34.0, 42.6, 52.3, 52.4, 60.9, 62.7, 68.2, 114.5, 129.3, 134.8, 157.3, 162.9, 164.2; IR (NaCl, neat) 1691 cm^{-1}; HRMS (ESI-MS): m/z calcd for $C_{26}H_{43}N_2O_3$ $[M + H]^+$ 431.3274, found 431.3261.

(c) A solution of TFA (3 mL) in water (6 mL) was added dropwise to a solution of (2*S*,5*S*)-5-isopropyl-3,6-dimethoxy-2-(4′-heptyloxyphenethyl)-2-ethyl-2,5-dihydropyrazine (0.14 g, 0.33 mmol) in acetonitrile (6 mL). The solution was stirred at room temperature for 76 h after which the acetonitrile was removed under reduced pressure. The residue was diluted with water and neutralised with portions of solid sodium bicarbonate, then extracted with dichloromethane (×4). The organic extracts were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with 50% ethyl acetate/*n*-hexane, to afford methyl (2*S*)-2-amino-2-ethyl-4-(4′-heptyloxyphenyl)butanoate as a clear colourless oil (27 mg, 25%). $[\alpha]_D^{27.8}$ = +20 (0.5, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) 0.84–0.90 (m, 6H), 1.25–1.37 (m, 6H), 1.40–1.47 (m, 2H), 1.56–1.65 (m, 1H), 1.69 (br s, 2H), 1.71–1.86 (m, 4H), 1.99–2.06 (m, 1H), 2.39 (td, J = 12.6, 4.8 Hz, 1H), 2.62 (td, J = 12.6, 5.3 Hz, 1H), 3.71 (s, 3H), 3.91 (t, J = 6.6 Hz, 2H), 6.79–6.82 (m, 2H), 7.05–7.08 (m, 2H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 8.4, 14.2, 22.7,

26.1, 29.2, 29.4, 29.8, 31.9, 33.2, 42.0, 52.2, 61.7, 68.1, 114.6, 129.3, 133.6, 157.5, 177.6; IR (NaCl, neat) 1732, 3325, 3385 cm^{-1}; HRMS (ESI-MS): *m/z* calcd for $C_{20}H_{34}NO_3$ $[M + H]^+$ 336.2539, found 336.22530.

(d) Lithium aluminium hydride (5 mg, 0.13 mmol) was added as a solid in one portion to a solution of methyl (2*S*)-2-amino-2-ethyl-4-(4′-heptyloxyphenyl)butanoate (27 mg, 80.5 μmol) in freshly distilled THF (0.5 mL) at 0 °C. The solution was stirred at 0 °C for 20 min then the cold bath was removed and the solution stirred at room temperature for 1 h. The reaction was quenched with saturated aqueous sodium sulfate solution and the mixture was extracted with ethyl acetate (×4). The organic extracts were combined and washed with saturated aqueous sodium bicarbonate solution, water and brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with 1% methanol/3% triethylamine/dichloromethane, to afford the title compound **20** as a clear gum (15 mg, 60%). $[\alpha]_D^{26.9}$ = −2 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) 0.86–0.93 (m, 6H), 1.25–1.60 (m, 12H), 1.64–1.81 (m, 3H), 1.89 (br s, 2H), 2.47–2.58 (m, 2H), 2.37 (s, 2H), 3.92 (t, *J* = 6.6 Hz, 2H), 6.79–6.83 (m, 2H), 7.06–7.11 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 7.9, 14.2, 22.7, 26.2, 29.1, 29.17, 29.20, 29.5, 31.9, 38.7, 55.3, 68.0, 68.2, 114.6, 129.2, 134.3, 157.5; IR (NaCl, neat) 3355 cm^{-1}; HRMS (ESI-MS): *m/z* calcd for $C_{19}H_{34}NO_2$ $[M + H]^+$ 308.2589, found 308.2572.

(4*S*)-4-(4′-Heptyloxyphenethyl)-4-methyloxazolidin-2-one (22). (2*S*)-*t*-Butyl(1-hydroxy-4-(4′-heptyloxyphenyl)-2-methylbutan-2-yl) carbamate[10] (22 mg, 49 μmol), potassium hydroxide (11 mg, 0.20 mmol) and tosyl chloride (11 mg, 58 μmol) in diethyl ether (2 mL) was heated at reflux for 15 h. The solution was allowed to cool to room temperature and poured onto ice water. The mixture was extracted with diethyl ether (×3). The organic extracts were combined and washed with water and brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with 20% ethyl acetate/*n*-hexane, to afford the title compound **22** as a clear colourless gum (14 mg, 90%). $[\alpha]_D^{26.7}$ = +12 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.89 (t, *J* = 6.8 Hz, 3H), 1.26–1.47 (m, 8H), 1.39 (s, 3H), 1.71–1.81 (m, 2H), 1.83–1.89 (m, 2H), 2.58–2.63 (m, 2H), 3.91 (t, *J* = 6.6 Hz, 2H), 4.04 (d, *J* = 8.5 Hz, 1H), 4.15 (d, *J* = 8.5 Hz, 1H), 6.48 (br s, 1H), 6.78–6.83 (m, 2H), 7.04–7.08 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 14.2, 22.7, 25.9, 26.1, 29.2, 29.4, 29.5, 31.9, 42.6, 57.8, 68.1, 75.8, 114.7, 129.2, 132.7, 157.7, 159.6; IR (NaCl, neat) 1729, 1764, 3161, 3248 cm^{-1}; HRMS (ESI-MS): *m/z* calcd for $C_{19}H_{29}NO_3Na$ $[M + Na]^+$ 342.2045, found 342.2045.

Synthesis of (2*S*)-1-amino-4-(4′-heptyloxyphenyl)-2-methylbutan-2-ol (23). (a) A solution of aqueous sodium hydroxide (3.5 mL, 0.4 M, 1.40 mmol) was added dropwise to a solution of 2-(trimethylsilyl)ethyl carbamate (0.23 g, 1.40 mmol) in *n*-propanol (1 mL) at room temperature. 1,3-Dichloro-5,5-dimethylhydantoin (0.18 g, 0.93 mmol) was added as a solid in one portion, followed by a solution of $(DHQD)_2AQN$ (20 mg, 23.3 μmol) in *n*-propanol (2.1 mL) and a solution of 1-(heptyloxy)-4-(3′-methylbut-3′-enyl)benzene[11] (0.12 g, 0.47 mmol) in *n*-propanol (0.4 mL). The solution was stirred vigorously at room temperature until it became homogeneous, then potassium osmate dihydrate (7 mg, 19.0 μmol) was added as a solid in one portion. The solution immediately turned dark green and was stirred at room temperature for 24 h after which, it had turned light yellow. Sodium sulphite (0.59 g, 4.70 mmol) was added and the suspension was stirred for a further 10 min. The mixture was diluted with water and extracted with ethyl acetate (×3). The organic extracts were combined and washed with brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material was purified by flash chromatography on silica gel, eluting with dichloromethane, to remove any residual 2-(trimethylsilyl) ethyl carbamate, then 30% ethyl acetate/*n*-hexane, to afford 2-(trimethylsilyl)ethyl (2*S*)-(4-(4-heptyloxyphenyl)-1-hydroxy-2-methylbutan-2-yl)carbamate as a clear colourless oil (0.16 g, 77%). $[\alpha]_D^{21.0}$ = −2 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.04 (s, 9H), 0.89 (t, *J* = 6.8 Hz, 3H), 0.95–1.01 (m, 2H), 1.23 (s, 3H), 1.26–1.47 (m, 10H), 1.72–1.81 (m, 2H), 2.23 (br s, 1H), 2.61–2.67 (m, 2H), 3.15–3.28 (m, 2H), 3.92 (t, *J* = 6.6 Hz, 2H), 4.14–4.19 (m, 2H), 5.01 (br s, 1H), 6.80–6.83 (m, 2H), 7.08–7.10 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ −1.34, 14.2, 17.9, 22.7, 24.7, 26.2, 29.2, 29.3, 29.5, 31.9, 42.1, 50.6, 63.5, 68.2, 77.4, 114.7, 129.3, 134.1, 157.5, 157.9; IR (NaCl, neat) 1699, 3417 cm^{-1}; HRMS (ESI-MS): *m/z* calcd for $C_{24}H_{43}NO_4SiNa$ $[M + Na]^+$ 460.2859, found 460.2860.

(b) A solution of hydrochloric acid in diethyl ether (0.53 mL, 2 M, 1.06 mmol) was added dropwise to a solution 2-(trimethylsilyl)ethyl (2*S*)-(4-(4-heptyloxyphenyl)-1-hydroxy-2-methylbutan-2-yl)carbamate (92 mg, 0.21 mmol) in methanol (5 mL) at room temperature. The solution was stirred at room temperature for 5 h. then the solvent was removed under reduced pressure. The residue was dissolved in 1 M aqueous hydrochloric acid and extracted with diethyl ether (×3). The remaining aqueous layer was collected and neutralised with solid sodium bicarbonate and extracted with dichloromethane (×3). The organic extracts were combined and washed with brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with 1% triethylamine/5% methanol/dichloromethane, to afford the title compound **23** as a clear colourless gum (28 mg, 45%). $[\alpha]_D^{25.6}$ = −2 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.89 (t, *J* = 6.5 Hz, 3H), 1.20 (s, 3H), 1.26–1.44 (m, 8H), 1.71–1.80 (m, 4H), 2.61–2.71 (m, 4H), 2.95 (br s, 3H), 3.91 (t, *J* = 6.5 Hz, 2H), 6.79–6.82 (m, 2H), 7.08–7.11 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 14.2, 22.7, 24.3, 26.2, 29.2, 29.3, 29.5, 31.9, 42.0, 50.7, 68.2, 71.4, 114.6, 129.3, 134.4, 157.4; IR (NaCl, neat) 3298, 3362 cm^{-1}; HRMS (ESI-MS): *m/z* calcd for $C_{18}H_{32}NO_2$ $[M + H]^+$ 294.2433, found 294.2432.

Synthesis of 2-amino-2-(4′-heptyloxyphenethyl)propane-1,3-diol (O-FTY720 (24)).[8b] (a) Cesium fluoride (0.32 g, 2.09 mmol) was added as a solid in one portion to a solution of diethyl 2-acetamido-2-(4′-(*t*-butyldimethylsilyloxy)phenethyl)malonate[17]

(**26**) (0.63 g, 1.39 mmol) in dry DMF (14 mL) at room temperature. The solution was stirred for 15 min, whereupon it turned dark orange, before 1-bromoheptane (0.24 mL, 1.53 mmol) was added dropwise. The solution was stirred at room temperature for 17 h. Water was added and the mixture was extracted with ethyl acetate (×3). The organic extracts were combined and washed with water and brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material purified by flash chromatography on silica gel, eluting with 50% ethyl acetate/*n*-hexane, to afford diethyl 2-acetamido-2-(4′-(*t*-butyldimethylsilyloxy)phenethyl) malonate as a white solid (0.57 g, 94%) with all the analytical data matching that reported in the literature.[17] Mp 79–81 °C; ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.89 (t, J = 6.8 Hz, 3H), 1.24 (t, J = 7.3 Hz, 6H), 1.28–1.48 (m, 8H), 1.71–1.80 (m, 2H), 1.99 (s, 3H), 2.39–2.44 (m, 2H), 2.62–2.68 (m, 2H), 3.90 (t, J = 6.6 Hz, 2H), 4.16–4.24 (m, 4H), 6.76 (br s, 1H), 6.78–6.82 (m, 2H), 7.01–7.06 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 14.1, 14.2, 22.8, 23.2, 26.1, 29.2, 29.37, 29.44, 31.9, 33.7, 62.7, 66.5, 68.2, 114.6, 129.4, 132.5, 157.7, 168.2, 169.1.

(b) Lithium aluminium hydride (78 mg, 2.06 mmol) was added as a solid in one portion to a solution of diethyl 2-acetamido-2-(4′-(*t*-butyldimethylsilyloxy)phenethyl)malonate (0.36 g, 0.84 mmol) in freshly distilled THF (4 mL) at 0 °C. The solution was stirred at 0 °C for 20 min then the cold bath was removed and the solution stirred at room temperature for 1 h. The reaction was quenched with saturated aqueous sodium sulfate solution and the mixture was extracted with ethyl acetate (×4). The organic extracts were combined and washed with saturated aqueous sodium bicarbonate solution, water and brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material used in the next step without any further purification. The crude material was dissolved in ethanol (4 mL) and concentrated hydrochloric acid solution (32%, 1 mL) was added dropwise. The mixture was heated at reflux for 16 h. The solution was allowed to cool to room temperature and the ethanol was removed under reduced pressure. The residue was neutralised with solid sodium bicarbonate and extracted with ethyl acetate (×4). The organic extracts were combined and washed with brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure to afford a brown gum which was recrystallised from ethanol to afford the title compound **24** as a white solid (0.16 g, 62%) with all the analytical data matching that reported in the literature.[8] ^{1}H NMR (300 MHz; MeOD) δ 0.91 (t, J = 6.7 Hz, 3H), 1.33–1.50 (m, 8H), 1.65–1.76 (m, 4H), 2.55–2.61 (m, 2H), 3.48 (d, J = 11.0 Hz, 2H), 3.54 (d, J = 11.0 Hz, 2H), 3.91 (t, J = 6.4 Hz, 2H), 6.78–6.81 (m, 2H), 7.10–7.13 (m, 2H); ^{13}C NMR (75 MHz; MeOD) δ 19.0, 26.7, 29.49, 29.53, 29.6, 30.3, 66.4, 68.9, 69.5, 85.0, 115.47, 115.51, 130.2, 135.9, 158.9.

4-(2-((2*S*,5*S*)-5-Isopropyl-3,6-dimethoxy-2-methyl-2,5-dihydropyrazin-2-yl)ethyl)phenyl trifluoromethanesulfonate (28). Triflic anhydride (0.05 mL, 0.30 mmol) was added dropwise to a solution of phenol **27**[10] (90 mg, 0.27 mmol) and pyridine (0.11 mL, 1.37 mmol) in dichloromethane (1.2 mL) at 0 °C. The solution was stirred at 0 °C for 1 hour then quenched with saturated aqueous sodium bicarbonate solution. The mixture was extracted with dichloromethane (×3). The organic extracts were combined and washed with saturated aqueous sodium bicarbonate solution, water and brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material was purified by flash chromatography on silica gel, eluting with 3% ethyl acetate/*n*-hexane, to afford the title compound **28** as a clear colourless oil (56 mg, 46%). $[\alpha]_D^{25.3}$ = + 48 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.69 (d, J = 6.8 Hz, 3H), 1.12 (d, J = 6.8 Hz, 3H), 1.31 (s, 3H), 1.87 (td, J = 12.8, 5.0 Hz, 1H), 2.10 (td, J = 12.8, 4.6 Hz, 1H), 2.28–2.43 (m, 2H), 2.51 (td, J = 12.8, 5.0 Hz, 1H), 3.69 (s, 3H), 3.71 (s, 3H), 3.94 (d, J = 3.3 Hz, 1H), 7.14–7.16 (m, 2H), 7.21–7.26 (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 17.0, 19.7, 28.6, 30.6, 31.1, 42.3, 52.46, 52.48, 58.3, 60.5, 118.9 (q, J_{C-F} = 318.9 Hz), 121.2, 130.2, 143.5, 147.8, 162.5, 165.4; IR (NaCl, neat) 1692 cm^{-1}; HRMS (ESI-MS): m/z calcd for $C_{19}H_{26}F_3N_2O_5S$ $[M + H]^+$ 451.1515, found 451.1451.

Synthesis of (2*S*)-2-amino-2-methyl-4-(4′-octylphenyl)butan-1-ol (25). (a) A solution of *n*-butyllithium in hexanes (1.2 mL, 1.4 M, 1.64 mmol) was added dropwise to a solution of freshly distilled (*S*)-Schöllkopf's reagent **29** (0.31 g, 1.64 mmol) in freshly distilled THF (3.2 mL) at −78 °C (dry ice/acetone). The solution was stirred at −78 °C for 15 min, where it had turned dark yellow. A solution of 1-(2-iodoethyl)-4-octylbenzene (**30**)[11] (0.53 g, 1.55 mmol) in freshly distilled THF (3.2 mL) at −78 °C was added dropwise. The solution was stirred for a further 30 min at −78 °C then allowed to slowly warm to −15 °C over 4 h. The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution and allowed to warm to room temperature. The THF was removed under reduced pressure and the residue extracted with dichloromethane (×4). The organic extracts were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material was purified by flash chromatography on silica gel, eluting with 2% ethyl acetate/*n*-hexane, to afford (2*R*,5*S*)-5-isopropyl-3,6-dimethoxy-2-(4-octylphenethyl)-2,5-dihydro-pyrazine as a clear colourless oil (0.52 g, 83%). $[\alpha]_D^{26.8}$ = −8 (0.5, $CHCl_3$); ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.70 (d, J = 6.8 Hz, 3H), 0.88 (t, J = 6.6 Hz, 3H), 1.06 (d, J = 6.8 Hz, 3H), 1.27–1.35 (m, 10H), 1.54–1.61 (m, 2H), 1.93–2.05 (m, 1H), 2.10–2.21 (m, 1H), 2.23–2.33 (m, 1H), 2.50–2.66 (m, 4H), 3.70 (s, 6H), 3.97 (t, J = 3.5 Hz, 1H), 4.06 (dt, J = 6.8, 3.5 Hz, 1H), 7.06–7.12 (m, 4H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 14.3, 16.8, 19.2, 22.8, 29.4, 29.5, 29.6, 30.7, 31.8, 31.9, 32.0, 35.7, 36.0, 52.50, 52.53, 55.2, 61.0, 128.4, 128.5, 139.4, 140.4, 163.7, 163.9; IR (NaCl, neat) 1695 cm^{-1}; HRMS (ESI-MS): m/z calcd for $C_{25}H_{41}N_2O_2$ $[M + H]^+$ 401.3168, found 401.3152.

(b) A solution of *n*-butyllithium in hexanes (0.9 mL, 1.4 M, 1.30 mmol) was added dropwise to a solution of (2*R*,5*S*)-5-isopropyl-3,6-dimethoxy-2-(4-octylphenethyl)-2,5-dihydropyrazine (0.52 g, 1.30 mmol) in freshly distilled THF (6.5 mL) at −78 °C (dry ice/acetone). The solution was stirred at −78 °C for 15 min, where it had turned dark yellow. Methyl iodide (0.16 mL, 2.57 mmol) was added dropwise. The solution was

stirred for a further 30 min at −78 °C then allowed to slowly warm to −15 °C over 4 h. The reaction mixture was quenched with saturated aqueous sodium bicarbonate solution and allowed to warm to room temperature. The THF was removed under reduced pressure and the residue extracted with dichloromethane (×4). The organic extracts were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material was purified by flash chromatography on silica gel, eluting with 2% ethyl acetate/*n*-hexane, to afford bis-lactim ether **31** as a clear colourless oil (0.28 g, 52%). $[\alpha]_D^{26.7}$ = +45 (0.5, $CHCl_3$); ^{1}H NMR (500 MHz; $CDCl_3$) δ 0.70 (d, J = 6.8 Hz, 3H), 0.88 (t, J = 7.2 Hz, 3H), 1.12 (d, J = 6.8 Hz, 3H), 1.27–1.33 (m, 10H), 1.31 (s, 3H), 1.55–1.62 (m, 2H), 1.88 (td, J = 13.0, 4.9 Hz, 1H), 2.11 (td, J = 13.0, 4.3 Hz, 1H), 2.28 (td, J = 13.0, 4.8 Hz, 1H), 2.35–2.41 (m, 1H), 2.46 (td, J = 13.0, 4.8 Hz, 1H), 2.54–2.59 (m, 2H), 3.70 (s, 3H), 3.71 (s, 3H), 3.94 (d, J = 3.3 Hz, 1H), 7.06–7.10 (m, 4H); ^{13}C NMR (125 MHz; $CDCl_3$) δ 14.3, 17.1, 19.7, 22.8, 28.7, 29.4, 29.5, 29.6, 30.7, 31.3, 31.8, 32.1, 35.7, 42.8, 52.4, 58.4, 60.5, 128.3, 128.4, 139.95, 140.33, 162.1, 165.7; IR (NaCl, neat) 1692 cm^{-1}; HRMS (ESI-MS): m/z calcd for $C_{26}H_{43}N_2O_2$ $[M + H]^+$ 415.3325, found 415.3273.

(c) A solution of TFA (3 mL) in water (6 mL) was added dropwise to a solution of bis-lactim ether **31** (0.28 g, 0.68 mmol) in acetonitrile (17 mL). The solution was stirred at room temperature for 4 h after which the acetonitrile was removed under reduced pressure. The residue was diluted with water and neutralised with portions of solid sodium bicarbonate, then extracted with dichloromethane (×4). The organic extracts were combined and dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material was purified by flash chromatography on silica gel, eluting with ethyl acetate, to afford methyl (2*S*)-2-amino-2-methyl-4-(4′-octylphenyl)butanoate as a clear colourless oil (0.14 g, 65%). $[\alpha]_D^{27.8}$ = +20 (0.5, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) 0.88 (t, J = 7.0 Hz, 3H), 1.26–1.33 (m, 10H), 1.37 (s, 3H), 1.54–1.62 (m, 2H), 1.84–1.91 (m, 1H), 1.99–2.06 (m, 1H), 2.44–2.66 (m, 4H), 3.70 (s, 3H), 7.06–7.10 (m, 4H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 14.2, 22.8, 26.7, 29.4, 29.5, 29.6, 30. 4, 31.7, 32.0, 35.7, 43.0, 52.3, 57.9, 128.3, 128.6, 138.8, 140.7, 178.2; IR (NaCl, neat) 1734, 3322, 3380 cm^{-1}; HRMS (ESI-MS): m/z calcd for $C_{20}H_{33}NO_2Na$ $[M + Na]^+$ 342.2409, found 342.2389.

(d) Lithium aluminium hydride (35 mg, 0.92 mmol) was added portion wise to a solution of methyl (2*S*)-2-amino-2-methyl-4-(4′-octylphenyl)butanoate (0.12 g, 0.37 mmol) in freshly distilled THF (3.7 mL) at 0 °C. The solution was stirred at 0 °C for 20 min then the cold bath was removed and the solution stirred at room temperature for 1 h. The reaction was quenched with saturated aqueous sodium sulfate solution and the mixture was extracted with ethyl acetate (×4). The organic extracts were combined and washed with saturated aqueous sodium bicarbonate solution, water and brine, then dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude material was purified by flash chromatography on silica gel, eluting with 6% methanol/0.5% triethylamine/dichloromethane, to afford the title compound **25** as a white solid (73 mg, 69%). Mp 93–95 °C; $[\alpha]_D^{25.2}$ = +6 (0.5, $CHCl_3$); ^{1}H NMR (400 MHz; $CDCl_3$) 0.88 (t, J = 7.0 Hz, 3H), 1.14 (s, 3H), 1.27–1.34 (m, 10H), 1.55–1.62 (m, 2H), 1.64–1.78 (m, 2H), 2.54–2.63 (m, 7H), 3.34–3.42 (m, 2H), 7.07–7.12 (m, 4H); ^{13}C NMR (125 MHz; $CDCl_3$) δ 14.2, 22.7, 24.2, 29.3, 29.4, 29.5, 29.9, 31.6, 31.9, 35.6, 41.7, 53.4, 69.7, 128.2, 128.5, 139.4, 140.4; IR (NaCl, neat) 3266, 3333 cm^{-1}; HRMS (ESI-MS): m/z calcd for $C_{19}H_{34}NO$ $[M + H]^+$ 292.2640, found 292.2625.

Biological data experimental

Cell lines. FDC.P1 mouse growth factor-dependent myeloid cell line expressing empty vector (EV) (maintained in 25 units per mL GM-CSF(GM)), and the oncogenic D816V mutant form of human c-KIT (previously described by Frost[18]) were used to determine analog selectivity and capacity to activate PP2A.[5c] The expression of c-KIT was routinely monitored by flow cytometry.[18] FDC.P1 cells were maintained in DMEM containing 10% fetal calf serum (FCS), 2 mM L-glutamine and 25 mM HEPES.

Cell viability assays. Cell viability was determined following treatment for 48 h with 0–10 μM of our novel analogs using a resazurin cell viability assay, as previously described.[5c]

PP2A activity. Cells were treated with 2.5 μM AAL(S) analogs for 12 h. PP2A–C was immunoprecipitated from 150 μg protein lysate with the Precipitator™ magnetic bead based platform (Abnova) and phosphatase activity against a phosphopeptide (KRpTIRR) was determined as previously described.[5c]

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Notes and references

1 J. A. Cohen, F. Barkhof, G. Comi, H.-P. Hartung, B. O. Khatri, X. Montalban, J. Pelletier, R. Capra, P. Gallo, G. Izquierdo, K. Tiel-Wilck, A. de Vera, J. Jin, T. Stites, S. Wu, S. Aradhye and L. Kappos, *N. Engl. J. Med.*, 2010, **362**, 402.

2 (*a*) V. Brinkmann, M. D. Davis, C. E. Heise, R. Albert, S. Cottens, R. Hof, C. Bruns, E. Prieschl, T. Baumruker, P. Hiestand, C. A. Foster, M. Zollinger and K. R. Lynch, *J. Biol. Chem.*, 2002, **277**, 21453; (*b*) V. Brinkmann, A. Billich, T. Baumruker, P. Heining, R. Schmouder, G. Francis, S. Aradhye and P. Burton, *Nat. Rev. Drug Discovery*, 2010, **9**, 883; (*c*) R. Albert, K. Hinterding, V. Brinkmann, D. Guerini, C. Müller-Hartweig, H. Knecht, C. Simeon, M. Streiff, T. Wagner, K. Welzenbach, F. Zécri, M. Zollinger, N. Cooke and E. Francotte, *J. Med. Chem.*, 2005, **48**, 5373.

3 L. Zhang, H.-D. Wang, X.-J. Ji, Z.-X. Cong, J.-H. Zhu and Y. Zhou, *Oncol. Rep.*, 2013, **30**, 2571.
4 For comprehensive recent reviews on the structure and function of PP2A see: (*a*) D. Perrotti and P. Neviani, *Lancet Oncol.*, 2013, **14**, e229; (*b*) M. Kiely and P. A. Kiely, *Cancers*, 2015, **7**, 648.
5 (*a*) P. Neviani, R. Santhanam, R. Trotta, M. Notari, B. W. Blaser, S. Liu, H. Mao, J. S. Chang, A. Galietta, A. Uttman, D. C. Roy, M. Valtieri, R. Brusner-Klisovic, M. A. Caligiuri, C. D. Bloomfield, G. Marcucci and D. Perotti, *Cancer Cell*, 2005, **8**(5), 355; (*b*) P. Neviani, R. Santhanam, J. J. Oaks, A. M. Eiring, M. Notari, B. W. Blaser, S. Liu, R. Trotta, N. Muthusamy, C. Gambacorti-Passerini, B. J. Druker, J. Cortes, G. Marcucci, C.-S. Chen, N. M. Verrills, D. C. Roy, M. A. Caligiuri, C. D. Bloomfield, J. C. Byrd and D. Perotti, *J. Clin. Invest.*, 2007, **117**(9), 2408; (*c*) K. G. Roberts, A. M. Smith, F. McDougall, H. Carpenter, M. Horan, P. Neviani, J. A. Powell, D. Thomas, M. A. Guthridge, D. Perrotti, A. T. R. Sim, L. K. Ashman and N. M. Verrills, *Cancer Res.*, 2010, **70**, 5438.
6 (*a*) C. A. Welsch, L. W. A. Roth, J. F. Goetschy and N. R. Movva, *J. Biol. Chem.*, 2004, **279**(35), 36720; (*b*) K. Romero Rosales, G. Singh, K. Wu, J. Chen, M. R. Janes, M. B. Lilly, E. R. Peralta, L. J. Siskind, M. J. Bennet, D. A. Fruman and A. L. Edinger, *Biochem. J.*, 2011, **439**, 299; (*c*) R. Fransson, A. N. McCracken, B. Chen, R. J. McMonigle, A. L. Edinger and S. Hanessian, *ACS Med. Chem. Lett.*, 2013, **4**, 969; (*d*) B. Chen, S. G. Roy, R. J. McMonigle, A. Keebaugh, A. N. McCracken, E. Selwan, R. Fransson, D. Fallegger, A. Huwiler, M. T. Kleinman, A. L. Edinger and S. Hanessian, *ACS Chem. Biol.*, 2016, **11**, 409.
7 (*a*) C. T. Wallington-Beddoe, J. Hewson, K. F. Bradstock and L. J. Bendall, *Autophagy*, 2011, **7**, 707; (*b*) Y. Yang, Q. Huang, Y. Lu, X. Li and S. Huang, *J. Cell. Biochem.*, 2012, **113**, 1314.
8 (*a*) M. Kiuchi, K. Adachi, T. Kohara, K. Teshima, Y. Masubuchi, T. Mishina and T. Fujita, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 101; (*b*) M. Kiuchi, K. Adachi, T. Kohara, M. Minoguchi, T. Hanano, Y. Aoki, T. Mishina, M. Arita, N. Nakao, M. Ohtsuki, Y. Hoshino, K. Teshima, K. Chiba, S. Sasaki and T. Fujita, *J. Med. Chem.*, 2000, **43**, 2946; (*c*) E. Jary, T. Bee, S. R. Walker, S. Chung, K. Seo, J. C. Morris and A. S. Don, *Mol. Pharm.*, 2010, **78**, 685; (*d*) A. Collison, L. Hatchwell, N. M. Verrills, P. A. B. Wark, A. P. de Siqueira, M. Tooze, H. Carpenter, A. S. Don, J. C. Morris, N. Zimmerman, N. W. Bartlett, M. E. Rothenberg, S. L. Johnston, P. S. Foster and J. Mattes, *Nat. Med.*, 2013, **19**, 232.
9 H. D. Toop, A. S. Don and J. C. Morris, *Org. Biomol. Chem.*, 2015, **13**, 11593.
10 K. Högenauer, K. Hinterding and P. Nussbaumer, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 1485.
11 See the ESI† for more details.
12 J. J. Ritter and P. P. Minieri, *J. Am. Chem. Soc.*, 1948, **70**, 4045.
13 E. N. Jacobsen, I. Marko, W. S. Mungall, G. Schroeder and K. B. Sharpless, *J. Am. Chem. Soc.*, 1988, **110**, 1968.
14 (*a*) U. Schöllkopf, U. Groth and C. Deng, *Angew. Chem., Int. Ed. Engl.*, 1981, **20**, 798; (*b*) U. Schöllkopf, *Tetrahedron*, 1983, **39**, 2085; (*c*) K. Hinterding, R. Albert and S. Cottens, *Tetrahedron Lett.*, 2002, **43**, 8095.
15 K. Hinterding, S. Cottens, R. Albert, F. Zecri, P. Buehlmayer, C. Spanka, V. Brinkmann, P. Nussbaumer, P. Ettmayer, K. Hoegenauer, N. Gray and S. Pan, *Synthesis*, 2003, 1667.
16 (*a*) K. B. Sharpless, D. W. Patrick, L. K. Truesdale and S. A. Biller, *J. Am. Chem. Soc.*, 1975, **97**, 2305; (*b*) G. Li, H.-T. Chang and K. B. Sharpless, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 451; (*c*) J. A. Bodkin and M. D. McLeod, *J. Chem. Soc., Perkin Trans. 1*, 2002, 2733.
17 A. Zivkovic and H. Stark, *Tetrahedron Lett.*, 2010, **51**, 3769.
18 M. J. Frost, P. T. Ferrao, T. P. Hughes and L. K. Ashman, *Mol. Cancer Ther.*, 2002, **1**, 1115.

