Supplementary Figure 1: WEHI-345 and WEHI-540 are inhibitors of RIPK2. (a) Chemical structure of WEHI-540 (b) Models of WEHI-345 (cyan carbons) and WEHI-540 (peach carbons) docked into the putative ATP binding pocket of RIPK2 (white carbons). Displayed are the amino acids which interact with WEHI-345 and hydrogen bonds are highlighted as yellow dashed lines. WEHI-540 is not able to interact with amino acids Tyr97, Pro99 and Asn100 thus was predicted to be a less active RIPK2 inhibitor. (c) In vitro kinase assay using endogenous RIPK1 from immortalized mouse BMDMs. Nec-1 potently inhibited P-Thr autophosphorylation of RIPK1 while WEHI345 and WEHI540 did not alter autophosphorylation of RIPK1. Shown is one experiment of at least 3 independent repeats (d) SV40 immortalized mouse dermal fibroblasts were either left untreated or treated with TNF, SMAC mimetic (Compound A, cA) and QVD to induce necroptosis. Addition of Nec-1 reduced the amount of cell death but WEHI345 or WEHI540 did not alter cell death. n = 6. In all panels, * indicate p values < 0.05 and ** indicate p values < 0.005 based on a non-paired Student’s t test. Error bars are SEM.
**Supplementary Figure 2**: WEHI-345 blocks cytokine transcription and secretion upon NOD stimulation and bacterial infection (a,b) THP-1 cells were stimulated with L18-MDP in the presence or absence of WEHI-345 (2 μM) and mRNA levels of IL-1β (a) and A20 (b) were determined using RTPCR, n = 3. (c) IFNγ primed wild-type BMDMs (n = 6) were treated with increasing concentrations of WEHI-345 before the addition of MDP and levels of MCP-1 were measured using ELISA 24 hours after the addition of MDP. ripk2−/− BMDMs (n=1) were used as a negative control. (d,e) Raw 267.4 cells were either left untreated or pretreated WEHI-345 or WEHI-540 before the addition of MDP and levels of TNF and IL-6 were measured by ELISA. n=3. Error bars are SEM. (f,g) CCR2+ and CCR2− monocytes of either wild-type or ripk2−/− mice were infected with *Listeria monocytogenes* and levels of TNF (f) or MIP-1α (g) in the supernatant were measured using Bioplex analysis. n=4. In all panels, * indicate p values < 0.05 and ** indicate p values < 0.005 based on a non-paired Student’s t test for control vs drug treated samples of one time point or one concentration. Error bars are SEM.
Supplementary Figure 3: WEHI-345 blocks cytokine secretion in vivo and ameliorates EAE (a,b) C57Bl/6 mice were challenged for 4 hours with MDP after pretreatment with either PBS, vehicle or 25 mg kg\(^{-1}\) WEHI-345 and serum levels of TNF (a) and MCP-1 (b) were determined using ELISA. n = 4 (PBS) or n = 5 (vehicle, WEHI-345). Error bars are SEM. (c) EAE was induced in wild-type C57 Bl/6 mice. On day 9 after disease induction, mice were treated twice daily with 20 mg kg\(^{-1}\) WEHI-345. The clinical score of each mouse was assessed daily and mice were followed up to day 23 after disease induction. control: n = 4, vehicle: n = 8, WEHI-345: n = 5. The mean clinical score ± SEM of one experiment is shown. Relevant p values are indicated. Error bars represent SEM.
Supplementary Figure 4 (previous page): WEHI-345 reduces weight loss and cytokine secretion in EAE using a new formulation. (a) EAE was induced in wild-type C57 Bl/6 mice and mouse weights at day 11 post immunization indicate a trend towards improved health condition in WEHI-345 treated mice. control: n = 6, vehicle: n = 6, WEHI-345: n = 6. Shown are individual values, averages and error bars are SEM. untreated vs vehicle: p=0.489, untreated vs WEHI-345: p=0.074, vehicle vs WEHI-345: p=0.085 (b-i) Cytokine and chemokine levels in the spleen and in the serum were measured at day 11 after immunization in the animals as in (a). There is a trend towards reduced cytokine and chemokine production in WEHI-345 treated mice (white bars). control: n = 6, vehicle: n = 6, WEHI-345: n = 6. Shown is the average and error bars are SEM as well as p values for vehicle vs WEHI-345 in the spleen. In all panels, * indicate p values < 0.05 and ** indicate p values < 0.005 based on a non-paired Student’s t test. (j) Representative sections of the forebrain of untreated (control), vehicle treated or WEHI-345 treated mice at day 11 after immunization. Parenchymal cellular infiltrate is reduced in WEHI-345 treated mice compared to control or untreated animals. Scale bar = 500 μm.
Supplementary Figure 5: Original Western blots for images used in figures 1b, 5a and 5b. Blocks indicate specific bands in the main figure.
Supplementary figure 6

Supplementary Figure 6: Original Western blots and Ponceau staining for images used in figures 5c-5f. Blocks indicate specific bands in the main figure.
Supplementary Figure 7: Original Western blots for images used in figure 6a and supplementary figure 1c. Blocks indicate specific bands in the main figure.
Supplementary Table 1: Inhibition of kinases by WEHI-345. A KINOMEScan for 96 kinases was performed using WEHI-345 at a concentration of 1 μM. Listed is the % inhibition for each kinase. Kinases inhibited more than 95% are highlighted in bold.

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### Supplementary Table 2: Disease scores of the first repeat of the EAE experiment.

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**Supplementary Table 3:** Disease scores of the second repeat of the EAE experiment.
Supplementary Methods

Synthesis of WEHI-345 and WEHI-540

WEHI-345

\[
\text{Tert-butyl 2-(2-methyl-1-nitropropan-2-yl)hydrazinecarboxylate (1): } \text{tert-Butyl carbazate (2.61 g, 19.8 mmol) was added to mixture of 2,2-dimethyl-nitroethylene (2.0 g, 19.8 mmol) in 20 mL of 1:1 water/acetonitrile. After one hour, TLC (CH}_2\text{Cl}_2/\text{MeOH 90:10) indicated complete reaction. The reaction was diluted with water. The aqueous phase was washed three times with EtOAc. The combined organic phases were rinsed with water and brine, dried over Na}_2\text{SO}_4 \text{ and concentrated. The solid residue was purified on SiO}_2 \text{ using CH}_2\text{Cl}_2/\text{MeOH 95:5 to 90:10. A white solid was obtained (3.6 g, 78%).}
\]

\[\delta 6.12 \text{ (br s, 1H, NH}\text{Boc), 4.43 (s, 2H, CH}_2\text{NO}_2\text{), 1.49 (s, 9H, tBu), 1.26 (s, 6H, CH}_3\text{x2).}\]
Tert-butyl 2-(1-(((benzyloxy)carbonyl)amino)-2-methylpropan-2-yl)hydrazine carboxylate (2): Pd/C (10%, 240 mg) was added to a mixture of compound 1 (1 g, 4 mmol) and ammonium formate (1.16 g, 18.4 mmol) in 8 mL of dry methanol. The reaction was stirred at room temperature for 1 hour (strong gas evolution) after which time TLC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95:5) indicated complete consumption of starting material. The reaction was filtered through a pad of celite and the solids were rinsed with methanol. The filtrate was concentrated. The colourless oil was used in the next step without further purification.

1H NMR (ppm, CDCl<sub>3</sub>): δ 6.60 (br s, 1H, NH<sub>Boc</sub>), 3.03 (br s, 2H +H<sub>2</sub>O, NH<sub>2</sub>), 2.78 (s, 2H, CH<sub>2</sub>NO<sub>2</sub>), 1.47 (s, 9H, tBu), 1.16 (s, 6H, 2 × CH<sub>3</sub>).

LCMS (+esi): 204.1 (M+H<sup>+</sup>).

The amine was dissolved in 8 mL of anhydrous acetonitrile. Triethylamine (0.5 mL, 3.6 mmol) and N-(Benzyloxycarbonyloxy)succinimide (897 mg, 3.6 mmol) were successively added. The reaction was stirred at room temperature for 16 hours and then concentrated. The residue was taken into EtOAc and 10% citric acid. The aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with 10% citric acid, water and brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. A colourless oil was obtained that crystallised slowly upon standing. No further purification was required (1.2 g, 90% for two steps).

1H NMR (ppm, CDCl<sub>3</sub>): δ 7.28-7.32 (m, 5H, Aromatic CH), 6.22 (br s, 1H, NH), 5.09 (s, 2H, CH<sub>2</sub>Ph), 3.51 (br s, 1H, NH), 3.04 (d, J = 6.57 Hz, 2H, CH<sub>2</sub>NH), 1.43 (s, 9H, tBu), 1.01 (s, 6H, 2 × CH<sub>3</sub>).

13C NMR (ppm, CDCl<sub>3</sub>): δ 157.6, 157.2, 136.8, 128.5, 128.1, 128.0, 81.1, 66.7, 57.7, 46.4, 28.3, 22.9.
**Benzyl (2-(5-amino-4-cyano-3-(p-tolyl)-1H-pyrazol-1-yl)-2-methylpropyl)carbamate** (3): Compound 2 (310 mg, 0.92 mmol) was dissolved in 1 mL of dry acetonitrile. Tosic acid (875 mg, 4.6 mmol) was dissolved in 3 mL of dry acetonitrile. Molecular sieves were added to both solutions and stood for 30 minutes. The solution of tosic acid was then added the other mixture and the reaction was stirred at room temperature for 3 hours. After that time the reaction was filtered and the solid washed with acetonitrile and CH₂Cl₂. The filtrate was concentrated to afford a thick colourless oil. It was dissolved in 2 mL of ethanol. Triethylamine (640 L, 1.48 mmol) and 2-(methoxy-p-tolyl-methylene)-malononitrile (182 mg, 0.92 mmol) were successively added. The reaction was then heated at 60 °C for 3 hours. After cooling down, the reaction was concentrated. Water and EtOAc were then added and the aqueous phase was extracted three times with EtOAc. The combined organic layers were dried over Na₂SO₄ and concentrated. The oily residue obtained was purified by flash chromatography on SiO₂ using 100% CH₂Cl₂ then CH₂Cl₂/MeOH 99:1. A colourless oil that solidifies slowly overnight was obtained (270 mg, 73%).

![Chemical Structure Image]

**¹H NMR (ppm, CDCl₃):** δ 7.74 (m, 2H, 2×H₆), 7.31 (m, 5H, aromatic CH from CBz), 7.20 (m, 2H, 2×H₃), 5.59 (m, 1H, CH₂NH), 5.08 (s, 2H, CH₂O), 4.40 (br s, 2H, NH₂), 3.77 (m, 2H, CH₂NH), 2.36 (s, 3H, CH₃), 1.58 (s, 6H, 2 × CH₃).

**¹³C NMR (ppm, CDCl₃):** δ 157.0, 151.6, 149.1, 139.1, 136.3, 129.4, 128.6, 128.3, 128.2, 126.1, 115.2, 76.3, 67.0, 63.2, 50.6, 24.2, 21.4.

**LCMS (+esi):** 404.3 (M+H⁺).
**Benzyl (2-(4-amino-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropyl) carbamate**: Compound 3 (100 mg, 0.25 mmol) and formamidine acetate (108 mg, 1 mmol) were reacted at 150 °C in 0.4 mL of methoxyethanol. 3 more portions of formamidine acetate were added at one hour intervals to the reaction mixture. After that time, water and EtOAc were added. The aqueous phase was extracted three times with EtOAc. The combined organic phases were washed two times with water and brine, dried over Na₂SO₄ and concentrated. The thick yellowish oil obtained was purified by two successive flash chromatographies: first using CH₂Cl₂/MeOH 100:0 to 97:3 then CH₂Cl₂/EtOAc 70:30 to 50:50. A colourless oil was obtained which solidified slowly (76 mg, 71%).

![Chemical structure of Benzyl (2-(4-amino-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropyl) carbamate](image)

¹H NMR (ppm, CDCl₃): δ 8.23 (s, 1H, Hc), 7.45 (m, 2H, 2×Hb), 7.23-7.28 (m, 7H, 2×Hb and 5 aromatic CH from CBz), 5.94 (br s, 2H, NH₂), 5.82 (m, 1H, CH₂NH), 5.02 (s, 2H, CH₂O), 3.87 (m, 2H, CH₂NH), 2.38 (s, 3H, CH₃), 1.71 (s, 6H, 2×CH₃).

¹³C NMR (ppm, CDCl₃): δ 156.9, 156.8, 153.7, 152.5, 143.8, 139.5, 136.6, 130.2, 129.9, 128.5, 128.4, 128.2, 128.1, 99.2, 66.8, 64.2, 49.8, 25.0, 21.3.

LCMS (+esi): 431.3 (M+H⁺).

**1-(1-amino-2-methylpropan-2-yl)-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine hydrobromide (5)**: Compound 4 (85 mg, 0.2 mmol) was treated with 1 mL of 33% solution of HBr in glacial acetic acid. The reaction was stirred at room temperature for 1 hour. Dry ether was then added to the mixture leading to the formation of a precipitate. It was collected by filtration, rinsed thoroughly with Et₂O and dried. 50 mg of the
compound were redissolved in 50 mL of water and freeze dried for two days. A white solid was obtained.

\[
\begin{array}{c}
\text{CH}_3 \\
\text{H}_2\text{N} \\
\text{H}_3 \text{N} \\
\text{H}_b \\
\text{H}_a \\
\text{Br}^{-} \text{H}_2\text{N}
\end{array}
\]

\[^1\text{H} \text{NMR (ppm, DMSO-d}_6\): \delta 8.40 (s, 1H, \text{H}_c), 7.95 (\text{br s, 3H, NH}_3^+), 7.53 (\text{m, 2H, 2×H}_b), 7.33 (\text{m, 2H, 2×H}_a), 3.59 (\text{m, 2H, CH}_2\text{NH}_3^+), 2.34 (\text{s, 3H, CH}_3), 1.73 (\text{s, 6H, 2×CH}_3).}

\[^{13}\text{C} \text{NMR (ppm, DMSO-d}_6\): \delta 154.2, 152.8, 149.5, 144.9, 138.8, 129.8, 128.8, 128.3, 98.5, 61.4, 46.5, 24.8, 20.9.}

LCMS (+esi): 297 (M-Br').

\(N-(2-(4\text{-amino-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl})-2\text{-methylpropyl) isonicotinamide, WEHI-345, :}

Compound 5 (64 mg, 0.17 mmol) was neutralised by treating a suspension of the hydrobromide salt in CH\(_2\)Cl\(_2\) with saturated aqueous NaHCO\(_3\), drying over K\(_2\)CO\(_3\) and concentrating. The residue was dissolved in CH\(_2\)Cl\(_2\) and isonicotinic acid (10.4 mg, 0.089 mmol) followed by solid supported DCC (Novabiochem, 390 mg, 0.51 mmol) were added and the reaction was placed on an orbital shaker for 24 hrs. The reaction was then filtered through a pad of celite. The solids were rinsed with CH\(_2\)Cl\(_2\). Concentration afforded a white solid (4.9 mg, 14%).
1H NMR (ppm, CDCl3): δ 8.78-8.77 (m, 1H, CH₂N), 8.71 (d, J = 6.0 Hz, 2H, 2×Hc), 8.36 (s, 1H, Hc), 7.65-7.64 (d, J = 6.0 Hz, 2H, 2×Hd), 7.55-7.54 (m, 2H, 2×Hb), 7.36-7.35 (m, 2H, 2×Ha), 4.11-4.10 (m, 2H, CH₂NH), 2.44 (s, 3H, CH₃), 1.83 (s, 6H, 2×CH₃).

13C NMR (ppm, CDCl3): δ 165.4, 157.8, 154.1, 153.9, 150.5, 143.7, 141.8, 139.5, 130.1, 129.9, 128.3, 120.9, 99.4, 63.9, 49.0, 25.6, 21.3.

LCMS (+esi): 402.1 (M+H⁺), RT = 6.70 min.

HRMS (ES+) Calculated for C₂₂H₂₃N₇O (M+H): 402.2037; found 402.2038.

WEHI-540

1-(1-(dimethylamino)-2-methylpropan-2-yl)-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine, WEHI-540. Compound 5 (50 mg, 0.13 mmol) was dissolved in 275 µL of CH₂Cl₂. Acetic acid (55 µL), 37% formaldehyde (275 µL) and 550 µL of a 1 M solution of NaBH₃CN in THF were added. The reaction was stirred for 90 minutes. After
that time, the reaction was diluted with CH$_2$Cl$_2$ and water was added. The aqueous phase was extracted three times with CH$_2$Cl$_2$. The combined organic layers were washed with water and brine and dried over Na$_2$SO$_4$. The residue was purified by flash chromatography using CH$_2$Cl$_2$/EtOAc (70:30 to 30:70). A white solid was obtained (22 mg, 51%).

$^1$H NMR (ppm, CDCl$_3$): $\delta$ 8.32 (s, 1H, $H_c$), 7.57-7.55 (m, 2H, 2×$H_b$), 7.33-7.31 (m, 2H, 2×$H_a$), 5.61 (br s, 2H, NH$_2$), 3.63 (s, 2H, CH$_2$N), 2.44 (s, 3H, CH$_3$), 2.42 (s, 6H, N(CH$_3$)$_2$), 1.94 (s, 6H, 2×CH$_3$).

$^{13}$C NMR (ppm, CDCl$_3$): $\delta$ 158.0, 154.9 (CH and C), 142.9, 138.9, 130.5, 130.0, 128.5, 99.8, 69.6, 63.5, 47.2 (2×CH$_3$), 26.0 (2×CH$_3$), 21.3.

LCMS (+esi): 325.2 (M+H$^+$).

HRMS (ES+) Calculated for C$_{18}$H$_{24}$N$_6$ (M+H): 325.2141; found 325.2126.
Methyl 2-(2-(2-azidoethoxy)ethoxy)ethoxy)isonicotinate (S1): A mixture of methyl 2-hydroxyisonicotinate (KUOPION YLIOPISTO, Patent: WO2008/129129 A1) (220 mg, 1.44 mmol), 1-azido-2-(2-(2-iodoethoxy)ethoxy)ethane\(^1\) and K\(_2\)CO\(_3\) (199 mg, 1.44 mmol) in THF (4 mL) and DMF (4 mL) was heated to 80 \(^\circ\)C for 24 hours under a nitrogen atmosphere. The THF was then removed under reduced pressure and the residue was diluted with water (2 mL) and extracted with EtOAc (2 × 3 mL). The combined organic fractions were dried with MgSO\(_4\) and concentrated under reduced pressure and the residue was chromotographed on SiO\(_2\) (0:100 to 100:0, EtOAc/cyclohexane).
Concentration of the appropriate fractions ($R_f = 0.6, 50:50$ EtOAc/cyclohexane) afforded the title compound (S1) as a tan coloured oil (191 mg, 43%).

$1H$ NMR (ppm, CDCl$_3$): $\delta$ 8.26 (m, 1H, $H_a$), 7.42-7.40 (m, 1H, $H_b$), 7.37 (s, 1H, $H_c$), 4.53-4.51 (m, 2H, 2 x $H_d$), 3.94 (s, 3H, C$_3$H$_3$), 3.90-3.88 (m, 2H, 2 x $H_e$), 3.75-3.73 (m, 2H, 2 x $H_f$), 3.71-3.68 (m, 4H, 2 x $H_f$ and 2 x $H_g$), 3.41-3.39 (m, 2H, 2 x $H_i$).

2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)isonicotinic acid (S2): Compound S1 (185 mg, 0.60 mmol) was dissolved in MeOH (0.5 mL) and THF (4 mL) and LiOH (48 mg, 1.19 mmol) in H$_2$O (0.25 mL) was added. The mixture was stirred at room temperature for 2 hours. The mixture was concentrated to a yellow solid, suspended in water and acidified to pH = 1 with HCl (1 M). The mixture was then extracted with EtOAc (3 x 10 mL) then dried (MgSO$_4$), filtered and concentrated under reduced pressure to afford the title acid (S2) (57 mg, 32%) as a colourless solid.

$1H$ NMR (ppm, CDCl$_3$): $\delta$ 8.26 (d, $J = 5.2$ Hz, 1H, $H_a$), 7.43 (d, $J = 5.2$ Hz, 1H, $H_b$), 7.40 (s, 1H, $H_c$), 4.53-4.50 (m, 2H, 2 x $H_d$), 3.92-3.89 (m, 2H, 2 x $H_e$), 3.77-3.74 (m, 2H, 2 x $H_f$), 3.72-3.65 (m, 4H, 2 x $H_f$ and 2 x $H_g$), 3.39-3.36 (m, 2H, 2 x $H_i$).

$N$-(2-(4-Amino-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropyl)-2-(2-(2-azidoethoxy)ethoxy)ethoxy)isonicotinamide (S3): Compound S2 (57 mg, 0.19 mmol) was dissolved in DMF (2 mL, anhydrous) and EDCI (52 mg, 0.27 mmol) and DMAP (71 mg, 0.58 mmol) were added. After 10 minutes compound 5 (free base,
114 mg, 0.38 mmol) was added and the mixture was stirred at room temperature under nitrogen for 48 h. The reaction mixture was diluted with EtOAc (10 mL) and washed with water (3 × 2 mL) and the aqueous washings were extracted with EtOAc (2 × 2 mL). The organic fractions were combined, dried (MgSO₄), filtered and concentrated. The crude material was chromatographed (silica, 0-100% EtOAc) and concentration of the appropriate fractions (Rf = 0.6, 7% MeOH(NH₃)/DCM) afforded the title compound (S3) as a sticky, colourless solid (63 mg, 57%).

1H NMR (ppm, CDCl₃): δ 8.68 (t, J = 6.1 Hz, 1H, NH), 8.34 (s, 1H, H), 8.18 (d, J = 5.3 Hz, 1H, Hₕ), 7.55-7.54 (m, 2H, 2 x Hₖ), 7.34-7.33 (m, 2H, 2 x Hₗ), 7.20 (dt, J = 5.3, 0.7 Hz, 1H, Hₜ), 7.15 (s, 1H, Hₜ), 4.49-4.47 (m, 2H, 2 x H₉), 4.08 (d, J = 6.1 Hz, 2H, CH₂NH), 3.84-3.83 (m, 2H, 2 x H₈), 3.70-3.67 (m, 2H, 2 x H₉), 3.66-3.63 (m, 4H, 2 x Hₕ and 2 x H₉), 3.34-3.32 (m, 2H, 2 x H), 2.44 (s, 3H, CH₃), 1.83 (s, 6H, 2 x CH₃).

N-(2-(4-Amino-3-(p-tolyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-2-methylpropyl)-2-(2-(2-aminoethoxy)ethoxy)ethoxy)isonicotinamide (S4): Compound S3 (60 mg, 0.10 mmol) was dissolved in THF (0.5 mL) and H₂O (0.5 mL). Trimethylphosphine (157 μL, 0.16 mmol, 1 M solution in THF) was added and the mixture was stirred for 12 hours at room temperature. Toluene (5 mL) was then added and the mixture was concentrated under reduced pressure. The crude material was chromatographed (0-15%,
MeOH(NH$_3$)/DCM). Concentration of the appropriate fractions ($R_f = 0.2$, 10% MeOH(NH$_3$)/DCM) afforded the title compound (S4) as a colourless solid (59 mg, 99%).

$^1$H NMR (ppm, CDCl$_3$): $\delta$ 8.88 (t, $J = 6.0$ Hz, 1H, NH), 8.33 (s, 1H, $H_i$), 8.17 (d, $J = 5.3$ Hz, 1H, $H_a$), 7.53-7.52 (m, 2H, 2 x $H_k$), 7.32-7.31 (m, 2H, 2 x $H_l$), 7.22 (d, $J = 5.3$ Hz, 1H, $H_b$), 7.16 (s, 1H, $H_l$), 4.48-4.46 (m, 2H, 2 x $H_d$), 4.05 (d, $J = 6.0$ Hz, 2H, CH$_2$NH), 3.83-3.82 (m, 2H, 2 x $H_e$), 3.68-3.67 (m, 2H, 2 x $H_h$ or 2 x $H_g$), 3.62-3.60 (m, 2H, 2 x $H_g$ or 2 x $H_h$), 3.51-3.49 (m, 2H, 2 x $H_b$), 2.86-2.84 (m, 2H, 2 x $H_l$), 2.63 (br s, 2H, NH$_2$), 2.41 (s, 3H, CH$_3$), 1.80 (s, 6H, 2 x CH$_3$).

$^{13}$C NMR (ppm, CDCl$_3$): $\delta$ 165.5, 164.1, 158.2, 154.8, 154.0, 147.5, 145.0, 143.5, 139.2, 130.09, 130.02, 128.4, 114.6, 108.9, 99.5, 70.5, 70.2, 69.6, 65.4, 63.7, 49.0, 41.14, 25.70, 25.64, 21.3.

LCMS (+esi): 547.3 (M+H$^+$).

HRMS (ES+) Calculated for C$_{28}$H$_{36}$N$_8$O$_4$ (M+H): 549.2932; found 549.2935.
$^1$H NMR of key intermediates and final products

Compound (4), 300 MHz.
Compound (5), 300 Mhz
WEHI-345, 600 MHz
WEHI-540, 300 MHz
Supplementary References