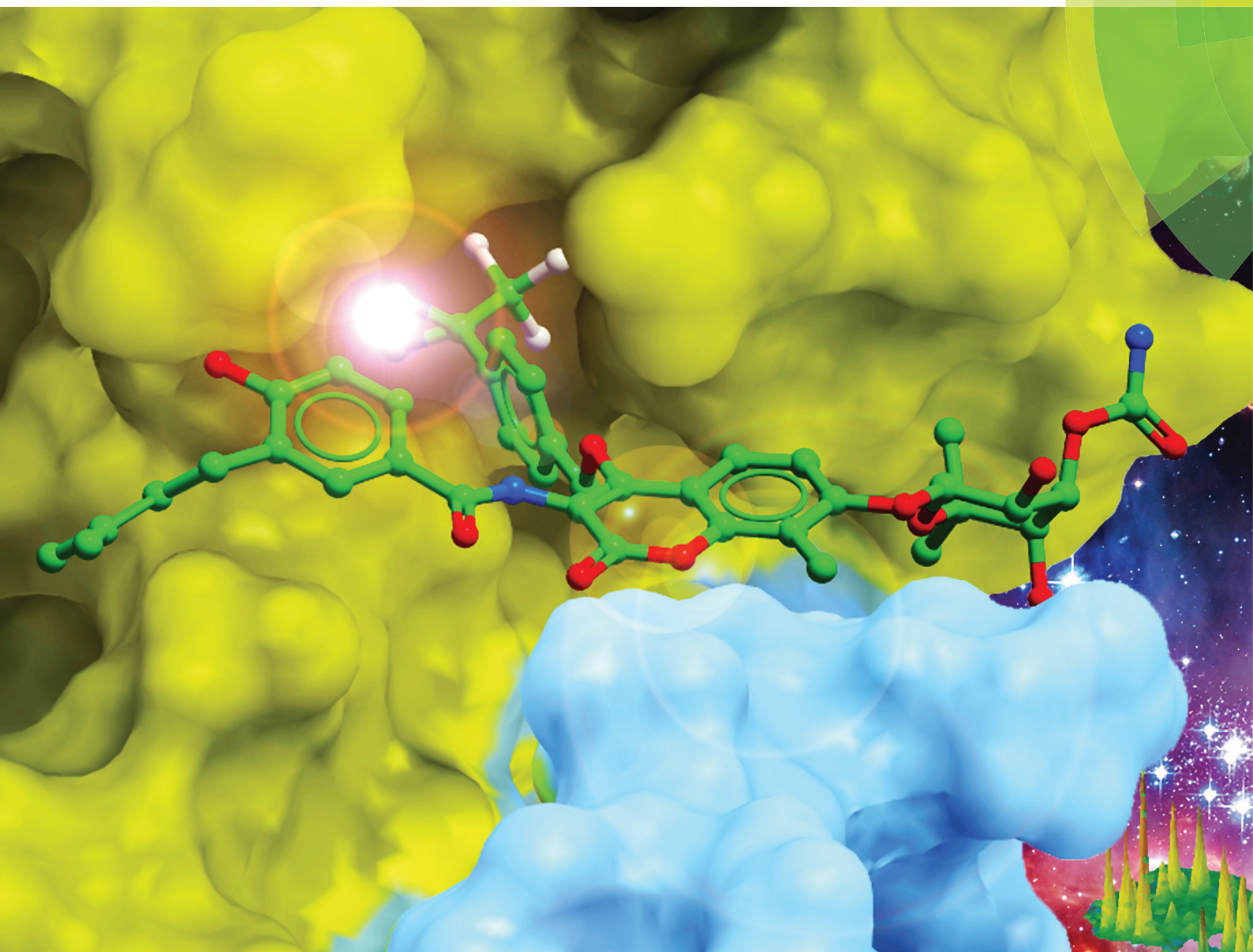


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PAPER

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Synthesis and characterization of photoaffinity labelling reagents towards the Hsp90 C-terminal domain



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Synthesis and characterization of photoaffinity labelling reagents towards the Hsp90 C-terminal domain†

Binto Simon,^a Xuexia Huang,^a Huangxian Ju,^b Guoxuan Sun^a and Min Yang^{*a}

Glucosyl-novobiocin-based diazirine photoaffinity labelling reagents (PALs) were designed and synthesized to probe the Hsp90 C-terminal domain unknown binding pocket and the structure–activity relationship. Five PALs were successfully synthesized from novobiocin in six consecutive steps employing phase transfer catalytic glycosylation. Reactions were monitored and guided by analytical LC/MS which led to different strategies of adding either a PAL precursor or a sugar moiety first. The structures and bonding linkages of these compounds were characterised by various 2D-NMR spectroscopy and MS techniques. Synthetic techniques provide powerful probes for unknown protein binding pockets.

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Introduction

Heat shock protein 90 kDa (Hsp90) is a molecular chaperone involved in folding up to 200 proteins with the assistance of 20 co-chaperones.¹ It has been the target of research in genetics and epigenetics,² neurodegeneration³ and cell mobility.⁴ Hsp90 is particularly important for cancer cell survival.^{5,6} So far seventeen Hsp90 inhibitors have been developed to various stages of clinical trials, however, candidates were suspended at different clinical stages.^{7,8} While these results validate Hsp90 inhibition as a relevant anti-cancer strategy, there is no FDA-approved drug^{1,9,10} due to the high cytotoxicity from N-terminal domain targeting inhibitors.

Hsp90 consists of three domains: an N-domain that contains an ATP, a drug-binding site and co-chaperone-interacting motifs; an M-domain for client proteins and co-chaperones; and a C-terminal domain (CTD) contains a dimerization MEEVD motif.¹¹ Inhibition of the Hsp90CTD decreases chaperone dimerization, diminishes ATPase activity and impairs the formation of the Hsp90 protein complex. Novobiocin based CTD inhibitors have shown some superior activity against various cancer cell lines in apoptosis¹² or proliferation¹³ compared to N-terminal inhibitors. Indeed, CTD inhibitors have attracted significant attention recently.^{10,14}

Due to the lack of a high resolution crystal structure, however, the binding site and structure–activity relationship (SAR) of the Hsp90CTD is still unclear. Most high resolution Hsp90 crystal structures do not contain a CTD. Only 4 Hsp90 crystals reported a low resolution CTD: 2IOQ (*E. coli*),¹⁵ 2CG9 (yeast),¹¹ 2O1U (dog)¹⁶ and 3Q6N (human, which has 18 amino acid residues missing in the CTD).¹⁷ Previous research indicated a few possible locations for the binding pockets^{18,19} in the CTD, however, no confirmation has been obtained. Although an experimental-based SAR can provide limited guidance in drug design (Fig. 1A), an in-depth understanding of the exact binding of ligand molecules to individual amino acid residues in the polypeptide is urgently required.

Previously we demonstrated that the single glycosylation of the 4'-hydroxyl group of novobiocin (Fig. 1A) could increase its anticancer activity^{20,21} via binding to the Hsp90CTD.²² Although protein modelling data indicate the formation of strong hydrogen bonds with Hsp90CTD peptides, the exact binding details is not clear.²⁰ We hereby designed and synthesized five glucosyl-novobiocin (Glc-Nov, Fig. 1B) based trifluoromethyl diazirine-type photoaffinity labelling reagents (PALs, 1–5) to probe the binding site of the Hsp90CTD.

These five compounds were designed to probe the binding of sugars (4'-glucose or 7'-noviose) with the Hsp90CTD. Compound 1 mimics Glc-Nov with the PAL at the 4'-OH position to probe the 4'-O-glucose moiety binding sites. Compound 2 mimics Glc-Nov with the PAL at the 7'-OH and glucose at the 4'-OH positions respectively. Compound 3 mimics novobiocin with the PAL at the 7'-OH position to probe the 7'-O-noviose moiety binding sites in the absence of the 4'-O-glucose moiety. Compound 4 tests the potential binding at the 2'-O position and compound 5 tests the binding site

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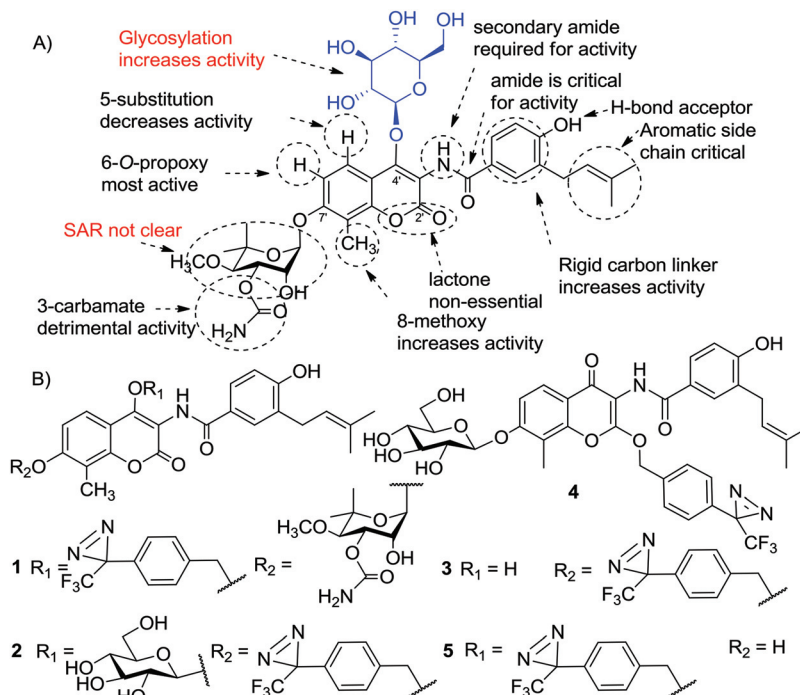


Fig. 1 Experimental based Hsp90CTD SAR (A) and five PALs (B).

with only the PAL at the 4'-O position, but no substitution on the 7'-OH group.

Trifluoromethyl diazirine has excellent chemical stability and is highly resistant toward a number of factors such as temperature, nucleophiles, acidic and basic conditions and oxidizing/reducing reagents. The activation by UV (350 nm) yields an extremely reactive flexible carbene, which can insert into C-H, N-H and O-H bonds²³ with low non-specific binding.²⁴

Results & discussion

The synthesis of compound **1** was achieved by reaction of novobiocin with diazirine bromide (**6**) in a polar aprotic solvent (DMF). Complete NMR assignment of all individual proton and carbon resonances has been achieved using ¹H-, ¹³C-NMR, DEPT-135, COSY, HSQC and HMBC (Fig. 2, S1a-c & Table S1†). The long range correlation between H1''' and the coumarin ring C4' in the HMBC spectrum indicates that C1''' was linked with the 4'-OH of novobiocin (Fig. 2).

The coumarin ring **7** (Table S2†) and 4-hydroxy-3(3-methylbutenyl)benzoic acid **8** (Table S3†) were obtained by acid hydrolysis of novobiocin following reported procedures, but with slight modification as shown in the Experimental section.²⁵ Coupling was performed using the Steglich esterification reaction employing 1-ethyl-3-(3-dimethylaminopropyl) to obtain aminocoumarin **9** with 62% yield. The NMR spectrum of **9** showed 4'-OH (br), 7'-OH and NH at 11.73, 10.52 and 9.44 ppm respectively (Fig. S2 and Table S4†). The long-range

heteronuclear (H-C) correlations between 7'-OH and C7', and C8' and C9' and NH coupled to C4' and C1 supported the assignment and also indicated the formation of the amide bond (Fig. S2c†).

Nucleophilic substitution reaction between compound **9** and the photoaffinity labelling reagent precursor **6** resulted in two compounds (Fig. 3, inset, S3 & S4†) with desired product masses (Fig. S3d, S3e, S4e and S4f†) of 4'- and 7'-linked products. Preparative HPLC indeed separated those two compounds, the one with a lower retention time (95 min, data not shown) was characterised to be the 4'-O compound **10** (Fig. 3, S3a-c and Table S5†) with the evidence of H1''' coupled to C4' in HMBC (Fig. S3b†). However, the fraction at 100 min was surprisingly not the expected 7'-O-compound as the NMR did not show evidence of linkage to the 7'-C position (Fig. S4b†). The 7'-OH and NH protons were still present in the ¹H-NMR spectrum (Fig. S4 and Table S6†). The spectrum showed a shift for H1''' from 3.3 ppm to 5.5 ppm and coupling to a carbon atom at a very similar position to C4' (Fig. S4d†), which was shown to be C2'. These results indicated that there is an equilibrium between the 4' and 2' positions in the coumarin ring and that compound **11** is linked to the 2'-OH in the enol form.

It is worth noting that conventional catalysts for glycosylation, such as Ag₂O, Ag₂CO₃, AgOTf, TMSOTf and Hg(CN)₂, did not work efficiently with glycosyl acceptors **10** and **11** (data not shown). Here we adopted a different route employing phase transfer glycosylation reaction. Optimised reaction conditions were obtained by conducting a number of experiments using varying reactant ratios, catalyst equivalents, base concentrations, organic solvents and even solid phase transfer

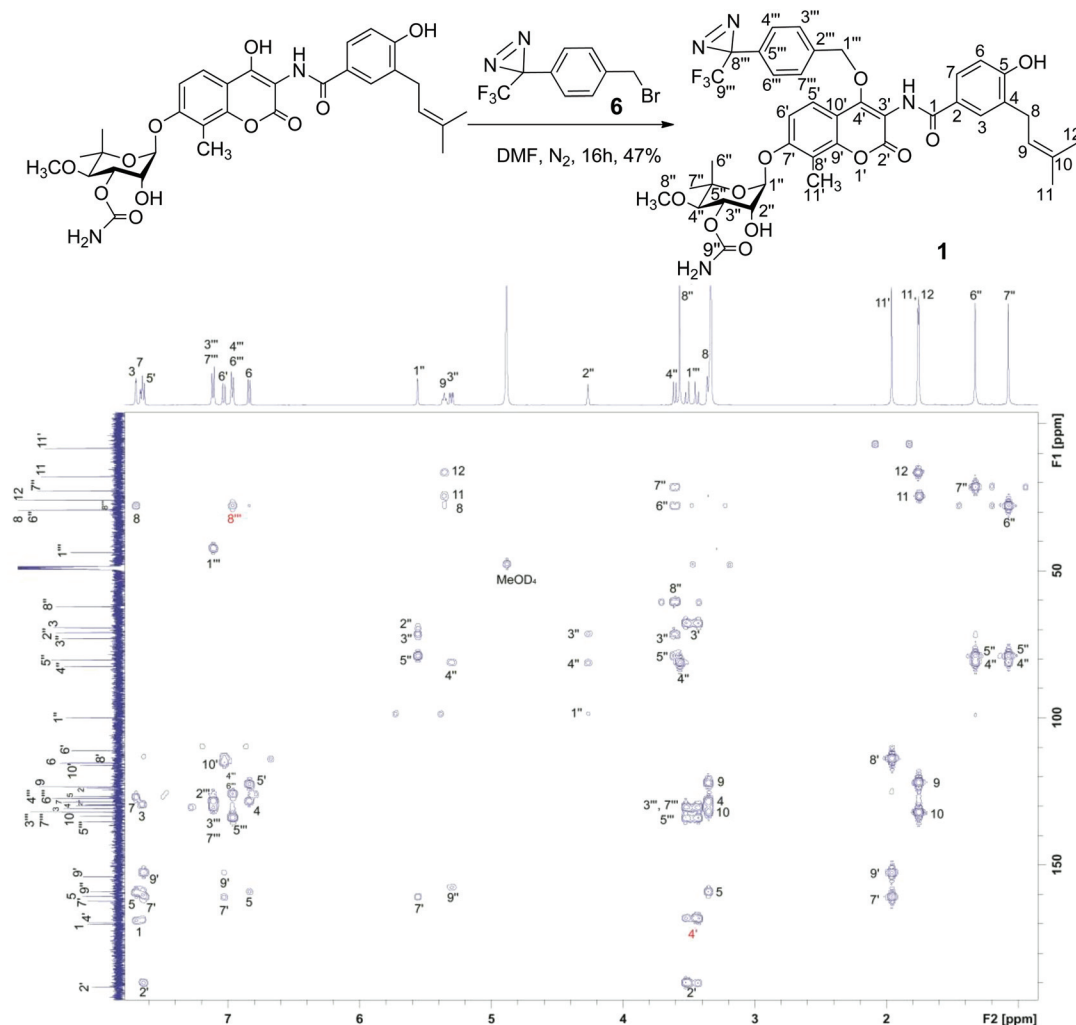


Fig. 2 Synthesis of compound **1**. Top: Synthesis scheme. Bottom: HMBC NMR assignment of compound **1**. Long range coupling between H1''' and C4' (red 4'). It also shows the coupling between C4'''/C6''' and C8''' (red 8''').

conditions (these investigations will be reported in future papers). Phase transfer glycosylation of compound **11** using benzyl-tri-butyl ammonium bromide (BTAB) gave **12** (Fig. S5 and Table S7†) with the glucose moiety linked with the 7'-OH of the coumarin ring. The disappearance of the 7'-OH signal and the coupling between H1''' and C7' demonstrated the correct assignment (Fig. S5a-c†). But, under the same conditions, glycosylation of compound **10** gave two different compounds (Fig. S6†) which are probably due to early activation of diazirine. Analytical LC/MS data showed that glycosylation happened but under such conditions, the compound lost N₂ to form a radical, which led to either an insertion reaction (938, [M + H]⁺, Fig. S6b†) or the removal of one additional acetyl group (896, [M + H]⁺, Fig. S6c†). Possible structures are proposed in Fig. S6b and S6c† based on the MS data and mechanism.

Since directly coupling compound **6** to the 7'-OH position of the coumarin was not possible, an alternative approach was

adopted by changing the sequence of reactions. The reaction of compound **9** with the sugar donor **13** gave the expected product **14** (Fig. 4, S7 and Table S8†) and a di-glucosyl product (data not shown). Compound **14** reacted with the PAL precursor **6** to give compound **15** in moderate yield (Fig. S8 and Table S9†).

Deprotection of the acetyl groups using NaOMe was slow, and step by step deacetylation was observed and confirmed by LC/MS, *e.g.* with one acetyl group (Fig. S9c & f†) four acetyl groups (Fig. S9c & g†) being observed. In addition, cleavage of the glycoside bond was also observed (Fig. S9c & h†) with the mass of 592 in negative mode probably due to unavoidable basic conditions caused by a trace amount of water. The deprotection of compound **15** gave the desired compound **2** (Fig. S9a-c and Table S10†) as well as compound **3** (Fig. S10 and Table S11†). Compounds **4** (Fig. S11 and Table S12†) and **5** (Fig. S12 and Table S13†) were obtained by deprotecting compounds **12** and **10**, respectively.

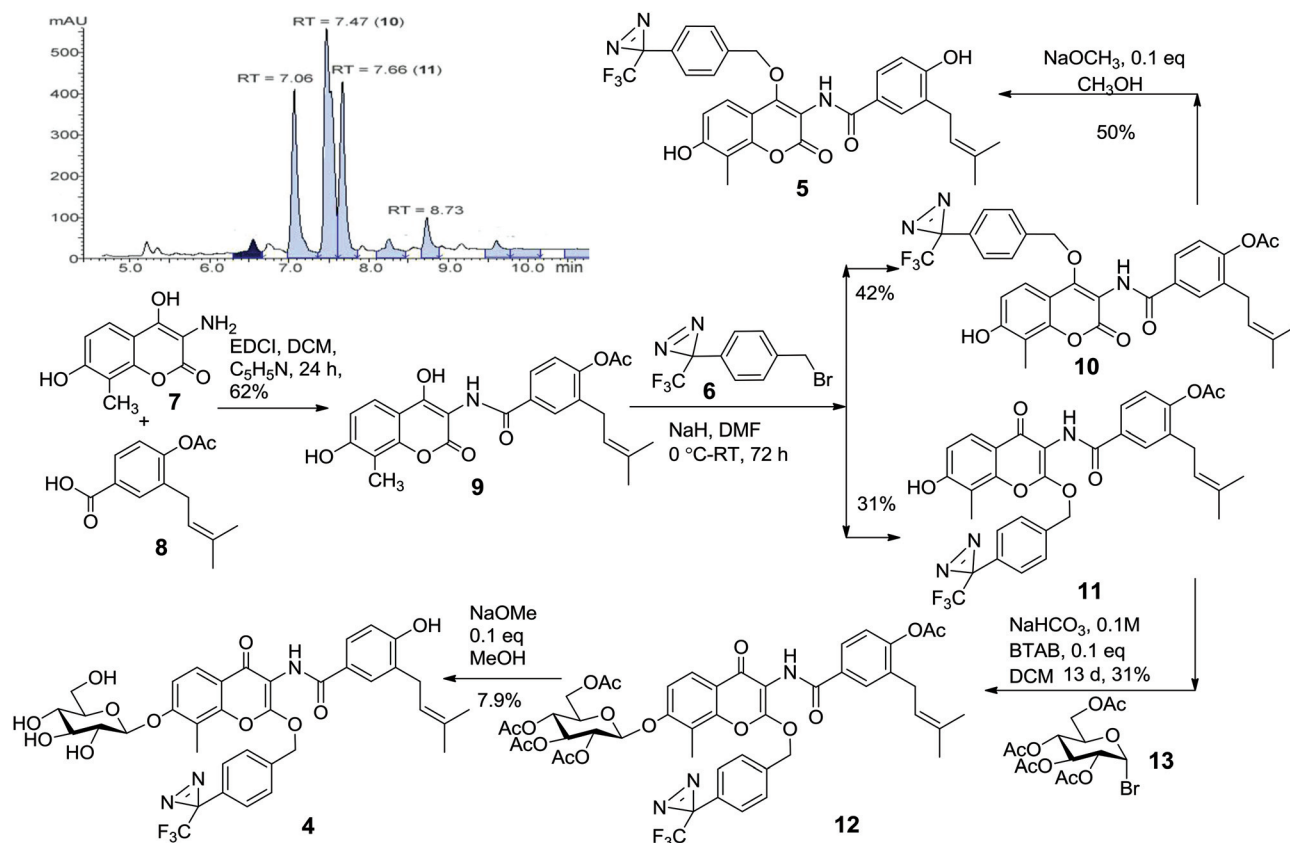


Fig. 3 Synthesis of compounds 4 & 5 (inset, top left, analytical LC-MS spectrum of compounds 10 & 11).

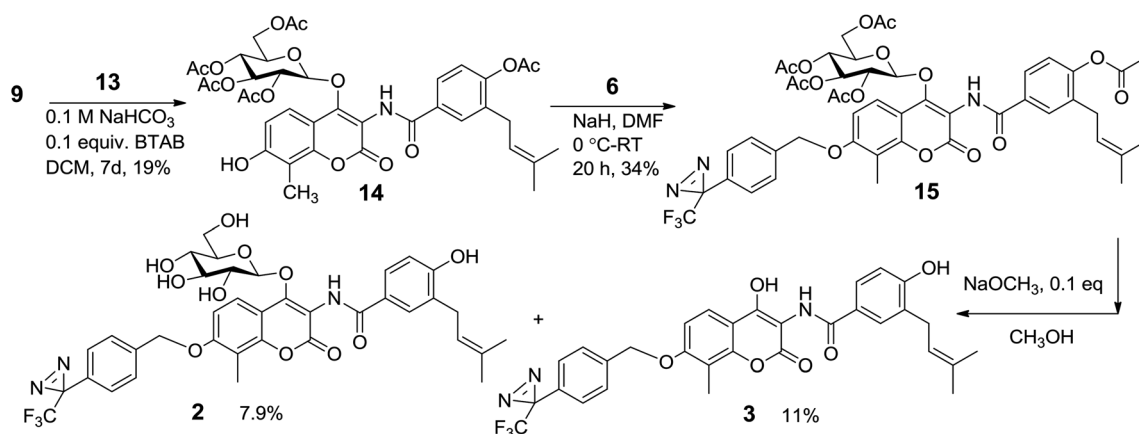


Fig. 4 Synthesis of compounds 2 & 3.

Conclusions

In conclusion, the synthesis of five glucosyl-novobiocin based PALs was achieved in six steps. This accentuates that the phase transfer glycosylation is an effective way for the incorporation of sugar moieties to molecules of interest with difficulties in conventional glycosylation methods. The structures have been confirmed by different NMR technologies. This strategy of

glucosyl-novobiocin modification provides a valuable approach for further development of enhanced glucosyl novobiocin mimetics. Preliminary data indicate the binding between the Hsp90CTD and compound 1, and tandem MS analysis indicated the exact peptide the PAL binds to. This method provides an effective synthetic route for multifunctional compounds, and a concise chemical biology tool to probe the unknown protein binding pocket SAR.

Experimental section

General experiment details

Unless otherwise stated, all reactions were carried out under anhydrous conditions. Reactions were monitored by thin layer chromatography (TLC) and/or a Shimadzu single quadrupole LC/MS. Flash column chromatography was performed on a Merck silica gel 60 (particle size 40–63 μm). ^1H NMR and ^{13}C NMR spectra were obtained using a Bruker 400 MHz or a Bruker 500 MHz spectrometer. Multiplicity is abbreviated as follows: (br = broad, s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, *etc.*) and coupling constants were obtained in hertz. Assignments were aided by DEPT 90, DEPT 135, COSY, NOESY, HSQC and HMBC. High resolution mass spectra were obtained using a Water Q-ToF MS. Chemicals were purchased from Sigma-Aldrich.

General procedure for phase transfer glycosylation

A brominated glycosyl donor (1.1–3.0 equiv.), a glycosyl acceptor (1 equiv.) and benzyl tributyl ammonium bromide (0.2 equiv.) in dichloromethane (DCM, 10 mL) were stirred for 5 min at room temperature. 0.1 M NaHCO_3 (6 mL) was then introduced to the reaction mixture. The reaction mixture was stirred for a specified time at room temperature. Upon completion (as indicated by TLC and LC/MS), the aqueous phase was washed with DCM (3 \times 30 mL). The dichloromethane extracts were combined and washed with saturated NaHCO_3 (2 \times 30 mL) and water (2 \times 30 mL) and dried (MgSO_4), filtered and solvents were removed *in vacuo* to give the corresponding glycosides. The crude material was purified by column chromatography (ethyl acetate : hexane 1 : 2 to 1 : 3 v : v depending on the product) then by preparative HPLC using methods 1–3.

HPLC purification methods

A Waters 2555 pump and a 2489 UV detector with a Waters C-18 (25 \times 200 mm) column. Solvent A 0.1% TFA in water, solvent B 0.1% TFA in MeOH. Flow rate: 10 mL min^{-1} . Three methods, HPLC-1 (100 min), HPLC-2 (175 min) and HPLC-3 (255 min).

HPLC-1: $t = 0$ min, 30% B, $t = 90$ min, 95% B, $t = 100$ min, 95% B, $t = 101$ min, 30% B, $t = 105$ min, 30% B.

HPLC-2: $t = 0$ min, 30% B, $t = 170$ min, 95% B, $t = 175$ min, 95% B, $t = 176$ min, 30% B, $t = 177$ min, 30% B.

HPLC-3: $t = 0$ min, 30% B, $t = 250$ min, 95% B, $t = 255$ min, 95% B, $t = 256$ min, 30% B, $t = 257$ min, 30% B.

Deprotection of acetate

To a solution of the acetylated compound (1 mmol) in dry methanol (10 mL vol. based on acetate groups) was added sodium methoxide (0.1 mmol). The mixture was stirred at room temperature and monitored by LC/MS. The reaction mixture was neutralised with a drop of acetic acid and then stirred for 0.5 h. The reaction mixture was filtered and concentrated under reduced pressure and then purified by preparative HPLC.

4-(4-(3-(Trifluoromethyl)-3H-diazirin-3-yl)phenyl)methanoyl-novobiocin (1)

The novobiocin sodium salt and 3-(4-(bromomethyl)phenyl)-3-(trifluoromethyl)-3H-diazirine (88 mg, 0.316 mmol) were dissolved in 5 mL of anhydrous DMF under the protection of nitrogen. The mixture was protected from light and stirred vigorously at room temperature for 16 h. The solvent was removed under vacuum and the syrup was purified by flash chromatography (ethyl acetate, $R_f = 0.5$) to yield compound **1** as a pale yellow solid (120 mg, 47%). $[\alpha]_D^{20}$: -130.5° (ACN). ^1H NMR (400 MHz, MeOD_4): $\delta = 7.55$ (1 H, s, H3), 6.69 (1 H, d, $J = 8.9$ Hz, H6), 7.51 (1 H, d, $J = 8.4$ Hz, H7), 3.21 (2 H, m, H8), 5.20 (1 H, t, $J = 7.4$ Hz, H9), 1.60 (6H, s, H11, H12), 7.50 (1 H, d, $J = 7.4$ Hz, H5'), 6.88 (1 H, d, $J = 8.4$ Hz, H6'), 1.81 (3 H, s, H11''), 5.41 (1 H, s, H1''), 4.12 (1 H, s, H2''), 5.15 (1 H, d, $J = 10.8$ Hz, H3''), 3.46 (1 H, d, $J = 10.8$ Hz, H4''), 1.18 (3 H, s, H6''), 0.93 (3 H, s, H7''); 3.42 (3 H, s, H8''); 3.33 (2 H, d, $J = 11.8, 36.9$ Hz, H1'''), 6.96 (2 H, d, $J = 7.9$ Hz, H3''', H7'''), 6.81 (2 H, d, $J = 7.9$ Hz, H4''', H6'''). ^{13}C NMR (125 MHz, MeOD_4): $\delta = 169.70$ (C1), 123.89 (C2), 130.91 (C3), 129.71 (C4), 160.6 (C5), 115.43 (C6), 128.51 (C7), 29.33 (C8), 123.40 (C9), 133.50 (C10), 26.10 (C11), 17.93 (C12), 191.50 (C2'), 69.34 (C3'), 170.30 (C4'), 126.60 (C5'), 111.25 (C6'), 162.50 (C7'), 115.33 (C8'), 154.10 (C9'), 116.21 (C10'), 8.30 (C11'), 100.07 (C1''), 71.01 (C2''), 72.96 (C3''), 82.55 (C4''), 80.34 (C5''), 29.34 (C6''), 22.92 (C7''), 62.08 (C8''), 159.17 (C9''), 43.90 (C11''), 127.34 (C2'''), 131.94 (C3'''), 129.72 (C4'''), 135.31 (C5'''), 127.34 (C6'''), 131.94 (C7'''), 29.10 (C8'''), 123.20 (C9'''). ESI^+ 811 $[\text{M} + \text{H}]^+$, HRMS: 811.2767, calculated for $\text{C}_{40}\text{H}_{42}\text{F}_3\text{N}_4\text{O}_{11}$: 811.2802.

3-Amino-4,7-dihydroxy-8-methyl-2H-chromen-2-one (7)

The novobiocin sodium salt (20 g, 0.0315 mol) was dissolved into a mixture of pyridine and acetic anhydride (5 : 1, 240 mL), and heated under reflux for 4 hours. After being cooled to room temperature, the mixture was then acidified with 5 N HCl drop by drop, the pH was monitored by using a pH paper (pH = 1) and the temperature of the reaction mixture was kept below 25 $^\circ\text{C}$ by using an ice bath. Then the brown syrup precipitated, and the aqueous phase was decanted. The brown syrup was washed with a small amount of diethyl ether (liquid kept for compound **8**), and later the precipitate of the crude product was collected. The crude product was further washed with diethyl ether again (liquid kept for compound **8**) until a light grey powder was obtained (9.5 g, 69%): $R_f = 0.13$, petroleum ether/ethyl acetate 1 : 2 v/v. The above solid (7.0 g, 0.03 mol) was dissolved in anhydrous methanol (105 mL) and 10% HCl/methanol (190 mL) and the mixture was refluxed for 2 h. The clear black solution obtained was evaporated *in vacuo* until precipitation started, and then the reaction mixture was kept at 4 $^\circ\text{C}$ overnight. The precipitate was filtered and washed with ice cold methanol to give the light grey powder. Then the filtrate was evaporated again and the procedure was repeated twice, and afforded a yellow solid (5.9 g, 95%). $R_f = 0.15$, chloroform : methanol 4 : 1 v/v. ^1H NMR (400 MHz, MeOD_4): $\delta = 7.77$ (1 H, d, $J = 8.03$ Hz, H5), 6.95 (1 H, d, $J = 8.56$ Hz, H6),

2.28 (3 H, s, H11). ^{13}C NMR (125 MHz, MeOD_4): δ = 164.2 (C2), 96.2 (C3), 161.7 (C4), 122.6 (C5), 113.7 (C6), 162.3 (C7), 113.5 (C8), 153.6 (C9), 108.1 (C10), 9.2 (C11). ESI^+ 208 $[\text{M} + \text{H}]^+$.

Synthesis of 4-acetoxy-3-(3-methylbut-2-en-1-yl)benzoic acid (8)

The filtrate from the above procedure was concentrated to afford a light brown syrup and was extracted using ethyl acetate (3 \times 25 ml) and with dilute hydrochloric acid (3 \times 20 ml of a 3% aqueous solution). The organic extracts were combined and washed sequentially with a saturated aqueous solution of NaHCO_3 (2 \times 20 ml) and water (2 \times 20 ml). The combined organic extracts were dried over magnesium sulphate, filtered and concentrated *in vacuo* and purified *via* column chromatography (petroleum ether:ethyl acetate 80:20) to afford a white crystalline powder (1.2 g, 16%). ^1H NMR (500 MHz, CDCl_3): δ = 8.03 (1H, s, H3), 7.17 (1H, d, J = 8.3 Hz, H6), 8.01 (1H, d, J = 8.3 Hz, H7), 3.31 (2H, d, J = 7.4 Hz, H8), 5.28 (1H, t, J = 7.1 Hz, H9), 1.79 (3H, s, H11), 1.75 (3H, s, H12), 2.34 (3H, s, H14). ^{13}C NMR (125 MHz, CDCl_3): δ = 171.8 (C1), 127.1 (C2), 132.3 (C-3), 129.3 (C4), 163.4 (C5), 123.6 (C6), 129.5 (C7), 29.3 (C8), 121.0 (C9), 134.1 (C10), 26.3 (C11), 17.7 (C12) 168.9 (C13), 21.0 (C14). ESI^+ 249 $[\text{M} + \text{H}]^+$.

Synthesis of 4-((4,7-dihydroxy-8-methyl-2-oxo-2H-chromen-3-yl)carbamoyl)-2-(3-methylbut-2-en-1-yl)phenyl acetate (9)

Compound 7 (1.0 g, 4.8 mmol) was dissolved in a mixture of anhydrous dichloromethane and pyridine (7:3). Then *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDCI, 1.0 g, 5.2 mmol) and compound 8 was added dropwise, dissolved in a mixture of DCM:pyridine (84:36) and stirred at room temperature under nitrogen. After 12 h, the reaction mixture was washed with aq. NaHCO_3 (30 ml) and water (30 mL \times 2), the organic phase was obtained and the excess solvent was removed *in vacuo* and the residue was purified *via* solid chromatography (petroleum ether:ethyl acetate, 3:1) to afford a yellow solid (1.3 g, 62%). ^1H NMR (500 MHz, DMSO-d_6): δ = 7.90 (1H, s, H3), 7.22 (1H, d, J = 8.1 Hz, H6), 7.89 (1H, d, J = 8.1 Hz, H7), 3.28 (2H, d, J = 7.1 Hz, H8), 5.22 (1H, t, J = 6.8 Hz, H9), 1.71 (6H, s, H11, H12), 2.33 (3H, s, H14), 7.61 (1H, d, J = 8.1 Hz, H5'), 6.90 (1H, d, J = 8.1 Hz, H6'), 2.19 (3H, s, H11'), 11.73 (1H, br, 4'-OH), 10.52 (1H, s, 7'-OH), 9.44 (1H, s, NH). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 165.94 (C1), 133.13 (C2), 129.96 (C3), 132.50 (C4), 150.99 (C5), 122.44 (C6), 126.94 (C7), 28.99 (C8), 121.55 (C9), 131.75 (C10), 26.36 (C11), 18.51 (C12), 168.89 (C13), 21.26 (C14), 160.57 (C2'), 99.72 (C3'), 160.85 (C4'), 121.52 (C5'), 111.83 (C6'), 159.10 (C7'), 110.43 (C8'), 151.47 (C9'), 108.07 (C10'), 8.88 (C11'). HMBC: NH linked with C4' and C1. ESI^+ 438 $[\text{M} + \text{H}]^+$. HRMS 438.1552, calculated 438.1553 ($\text{C}_{24}\text{H}_{24}\text{NO}_7$).

4-((7-Hydroxy-8-methyl-2-oxo-4-((4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)oxy)-2H-chromen-3-yl)carbamoyl)-2-(3-methylbut-2-en-1-yl)phenyl acetate (10)

To an oven dried flask equipped with a magnetic stirrer bar, rubber septa and a nitrogen inlet, compound 9 (0.2 g, 0.45 mmol) and 10 mL of anhydrous DMF were added. The

solution was cooled to 0 °C and sodium hydride (4.2 mg of 60% mineral oil dispersion) was added, and then the reaction mixture was allowed to stir for 30 min at 0 °C. Compound 6 (0.14 g, 0.49 mmol) dissolved in DMF was added dropwise to the reaction flask. The reaction was allowed to slowly warm up to room temperature and was stirred for 72 h. The reaction mixture was then extracted with ethyl acetate (2 \times 20 ml) and water. The combined organic extracts were dried over magnesium sulfate. The drying agent was filtered off and the organic extract was concentrated *in vacuo* to give the compound as a yellow foam. The desired products were isolated *via* preparative HPLC-1 with a retention time of 95 min and freeze dried to obtain a light yellow solid (0.12 g, 42%). ^1H NMR (500 MHz, DMSO-d_6): δ = 7.86 (1H, s, H3), 7.34 (1H, d, J = 8.2 Hz, H6), 7.92 (1H, d, J = 8.8 Hz, H7), 3.34 (2H, d, J = 7.6 Hz, H8), 5.28 (1H, t, J = 7.1 Hz, H9), 1.79 (3H, s, H11), 1.77 (3H, s, H12), 2.42 (3H, s, H14), 7.51 (1H, d, J = 9.5 Hz, H5'), 6.77 (1H, d, J = 8.9 Hz, H6'), 1.90 (3H, s, H11'), 3.4 (2H, m, H1''), 7.21 (2H, d, J = 7.6 Hz, H3'', H7''), 7.09 (2H, d, J = 7.6 Hz, H4'', H6''), 10.96 (1H, s, 7'-OH), 9.93 (1H, s, NH). ^{13}C NMR (125 MHz, DMSO-d_6): δ = 166.05 (C1), 129.46 (C2), 129.82 (C3), 133.61 (C4), 151.56 (C5), 122.84 (C6), 126.72 (C7), 28.92 (C8), 121.24 (C9), 132.80 (C10), 26.06 (C11), 18.45 (C12), 169.90 (C13), 8.19 (C14), 188.40 (C2'), 67.69 (C3'), 167.57 (C4'), 125.24 (C5'), 112.05 (C6'), 163.17 (C7'), 111.78 (C8'), 152.69 (C9'), 110.68 (C10'), 7.49 (C11'), 42.4 (C1''), 126.84 (C2''), 130.78 (C3''), 125.87 (C4''), 134.30 (C5''), 125.87 (C6''), 130.78 (C7''), 28.43 (C8''), 123.76 (C9''). HMBC: NH linked with C4' and C1. ESI^+ 636 $[\text{M} + \text{H}]^+$. HRMS: 636.1959, calculated for $\text{C}_{33}\text{H}_{29}\text{F}_3\text{N}_3\text{O}_7$: 636.1958.

4-((7-Hydroxy-8-methyl-4-oxo-2-((4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)oxy)-4H-chromen-3-yl)carbamoyl)-2-(3-methylbut-2-en-1-yl)phenyl acetate (11)

To an oven dried flask equipped with a magnetic stirrer bar, rubber septa and a nitrogen inlet, compound 9 (0.2 g, 0.45 mmol) and 10 mL of anhydrous DMF were added. The solution was cooled to 0 °C and sodium hydride (4.2 mg of 60% mineral oil dispersion) was added, and then the reaction mixture was allowed to stir for 30 min at 0 °C. Compound 6 (0.14 g, 0.49 mmol) dissolved in DMF was added dropwise to the reaction flask. The reaction was allowed to slowly warm up to room temperature and was stirred for 72 h. The reaction mixture was then extracted with ethyl acetate (2 \times 20 ml) and water. The combined organic extracts were dried over magnesium sulfate. The drying agent was filtered off and the organic extract was concentrated *in vacuo* to give the compound as a yellow foam. The desired products were isolated *via* preparative HPLC-1 with a retention time of 100 min and freeze dried to obtain a light yellow solid (0.09 g, 31%). ^1H NMR (500 MHz, DMSO-d_6): δ = 7.83 (1H, s, H3), 7.22 (1H, d, J = 8.8 Hz, H6), 7.82 (1H, d, J = 5.8 Hz, H7), 3.27 (2H, d, J = 7.4 Hz, H8), 5.20 (1H, t, J = 5.7 Hz, H9), 1.69 (6H, s, H11, H12), 2.33 (3H, s, H14), 7.50 (1H, d, J = 8.9 Hz, H5'), 6.90 (1H, d, J = 8.8 Hz, H6'), 2.19 (3H, s, H11'), 5.46 (2H, s, H1''), 7.55 (2H, d, J = 8.1 Hz, H3'', H7''), 7.27 (2H, d, J = 7.5 Hz, H4'', H6'').

^{13}C NMR (125 MHz, DMSO- d_6): δ = 166.23 (C1), 131.17 (C2), 129.70 (C3), 133.49 (C4), 151.38 (C5), 122.76 (C6), 126.62 (C7), 28.39 (C8), 121.34 (C9), 132.63 (C10), 25.36 (C11), 17.59 (C12), 168.90 (C13), 20.81 (C14), 160.97 (C2'), 48.71 (C3'), 161.16 (C4'), 121.75 (C5'), 112.27 (C6'), 159.37 (C7'), 108.52 (C8'), 150.83 (C9'), 110.66 (C10'), 8.12 (C11'), 73.10 (C1''), 127.50 (C2''), 128.52 (C3''), 126.62 (C4''), 138.46 (C5''), 126.62 (C6''), 128.52 (C7''), 28.56 (C8''), 124.0 (C9''). HMBC: H1'' linked with C2'. ESI⁺ 636 [M + H]⁺. HRMS: 636.1963, calculated for C₃₃H₂₉F₃N₃O₇: 636.1958.

(2S,3R,5R,6R)-2-((3-(4-Acetoxy-3-(3-methylbut-2-en-1-yl)benzamido)-8-methyl-4-oxo-2-((4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)oxy)-4H-chromen-7-yl)oxy)-6-(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (12)

Compound **11** (0.05 g, 0.07 mmol) was reacted with glucosyl bromide (compound **13**, 0.12 g, 0.30 mmol, 3 equiv.) using the general phase transfer glycosylation procedure (in DCM) for 13 days. The product was purified by preparative HPLC-2 with a retention time of 96 min to afford compound **12** as a colourless white powder (LCMS yield: 52%, isolated yield, 0.02 g, 31%). [α]_D²⁰: -22.41°, (CHCl₃). ^1H NMR (500 MHz, CDCl₃): δ = 7.88 (1H, d, J = 2.33 Hz, H3), 7.20 (1H, d, J = 8.33 Hz, H6), 7.82 (1H, dd, J = 2.46, 9.49 Hz, H7), 3.34 (2H, d, J = 7.02 Hz, H8), 5.25 (1H, m, H9), 1.79 (3H, s, H11), 1.75 (3H, s, H12), 2.38 (3H, s, H14), 7.70 (1H, d, J = 9.02 Hz, H5'), 7.01 (1H, d, J = 9.07 Hz, H6'), 2.29 (3H, s, H11''), 5.38 (2H, s, H1'''), 7.50 (2H, d, J = 8.60 Hz, H3'', H7''), 7.21 (2H, d, J = 8.21 Hz, H4'', H6''), 5.15 (1H, d, J = 7.68 Hz, H1'''), 5.39 (1H, m, H2'''), 5.36 (1H, m, H3'''), 5.24 (1H, m, H4'''), 3.93 (1H, m, H5'''), 4.34 (1H, m, H6a'''), 4.22 (1H, m, H6b'''), 2.09-2.11 (12 H, s, 4 × CH₃CO), 7.75 (1H, s, NH). ^{13}C NMR (125 MHz, CDCl₃): δ = 166.62 (C1), 130.82 (C2), 129.97 (C3), 134.78 (C4), 152.50 (C5), 123.11 (C6), 126.38 (C7), 28.92 (C8), 120.78 (C9), 134.27 (C10), 25.71 (C11), 17.90 (C12), 168.98 (C13), 20.82 (C14), 157.78 (C2'), 104.95 (C3'), 162.43 (C4'), 122.03 (C5'), 111.62 (C6'), 157.33 (C7'), 116.11 (C8'), 150.00 (C9'), 113.19 (C10'), 8.4 (C11'), 72.49 (C1''), 129.64 (C2''), 128.26 (C3''), 126.82 (C4''), 137.73 (C5''), 126.82 (C6''), 128.26 (C7''), 28.19 (C8''), 122.6 (C9''), 99.22 (C1'''), 70.89 (C2'''), 72.18 (C3'''), 68.22 (C4'''), 72.23 (C5'''), 62.01 (C6a'''), 62.01 (C6b'''), 20.69 (4 × CH₃CO), 170.51 (CH₃CO), 170.22 (CH₃CO), 169.41 (CH₃CO), 169.21 (CH₃CO). HMBC: H1'''' linked with C7' and H1'' linked with C2'. ESI⁺ 966 [M + H]⁺. HRMS: 966.2873, calculated for C₄₇H₄₇F₃N₃O₁₆: 966.2908.

Glycosylation of compound 10

The same procedure was followed in the synthesis of compound **12**, *i.e.* compound **10** (0.05 g, 0.07 mmol) was reacted with glucosyl bromide (compound **13**, 0.12 g, 0.30 mmol, 3 equiv.) using the general phase transfer glycosylation procedure (in DCM) for 13 days. However, no desired glycosylation product formed but new reactions with N₂ removal and possible insertions were discovered by LC/MS.

(2S,3R,5R,6R)-2-((3-(4-Acetoxy-3-(3-methylbut-2-en-1-yl)benzamido)-7-hydroxy-8-methyl-2-oxo-2H-chromen-4-yl)oxy)-6-(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (14)

Compound **9** (0.3 g, 0.68 mmol) was reacted with glucosyl bromide (**13**, 0.84 g, 2.0 mmol) using the general phase transfer glycosylation procedure (in DCM) for 7 days. The product was purified by preparative HPLC-2 with a retention time of 112 min to afford compound **14** as a white powder (0.10 g, 19%). [α]_D²⁰: -66.03°, (CHCl₃). ^1H NMR (500 MHz, CDCl₃): δ = 7.95 (1H, s, H3), 7.23 (1H, d, J = 9.15 Hz, H6), 7.87 (1H, d, J = 8.56 Hz, H7), 3.37 (2H, d, J = 7.85 Hz, H8), 5.27 (1H, m, H9), 1.79 (3H, s, H11), 1.76 (3H, s, H12), 2.38 (3H, s, H14), 7.35 (1H, d, J = 9.18 Hz, H5'), 6.75 (1H, d, J = 9.21 Hz, H6'), 2.21 (3H, s, H11''), 5.36 (1H, m, H1'''), 5.36 (1H, m, H2'''), 5.24 (1H, m, H3'''), 5.12 (1H, t, J = 9.39 Hz, H4'''), 3.64 (1H, m, H5'''), 4.17 (1H, m, H6a'''), 4.24 (1H, m, H6b'''), 2.00-2.07 (12 H, s, 4 × CH₃CO), 7.81 (1H, s, NH). ^{13}C NMR (125 MHz, CDCl₃): δ = 167.51 (C1), 130.57 (C2), 130.19 (C3), 134.87 (C4), 152.49 (C5), 123.00 (C6), 126.49 (C7), 29.05 (C8), 120.79 (C9), 134.06 (C10), 25.76 (C11), 17.96 (C12), 168.85 (C13), 20.55 (C14), 157.55 (C2'), 107.02 (C3'), 160.98 (C4'), 121.41 (C5'), 112.64 (C6'), 158.71 (C7'), 109.01 (C8'), 151.16 (C9'), 112.10 (C10'), 8.08 (C11'), 98.95 (C1''), 71.20 (C2''), 72.35 (C3''), 67.98 (C4''), 72.57 (C5'') 61.49 (C6''), 20.54-20.63 (4 × CH₃CO), 170.56 (CH₃CO), 170.05 (CH₃CO), 169.39 (CH₃CO), 169.33 (CH₃CO). ESI⁻ 766 [M - H]⁻. HRMS: 768.2504, calculated for C₃₈H₄₂NO₁₆: 768.2504.

(3R,5R,6R)-2-((3-(4-Acetoxy-3-(3-methylbut-2-en-1-yl)benzamido)-8-methyl-2-oxo-7-((4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzyl)oxy)-2H-chromen-4-yl)oxy)-6-(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (15)

To an oven dried flask equipped with a magnetic stirrer bar, rubber septa and a nitrogen inlet, compound **14** (0.05 g, 0.07 mmol) and 10 mL of anhydrous DMF were added. The solution was cooled to 0 °C and sodium hydride (2.1 mg of 60% mineral oil dispersion) was added, and then the reaction mixture was allowed to stir for 30 min at 0 °C. Compound **6** (0.02 g, 0.08 mmol) dissolved in DMF was added dropwise to the reaction flask. The reaction was allowed to slowly warm up to room temperature and was stirred for 20 h. The reaction mixture was then extracted with ethyl acetate (2 × 20 ml) and water. The combined organic extracts were dried over magnesium sulfate. The drying agent was filtered off and the organic extract was concentrated *in vacuo* to give the compound as a yellow foam. The desired products were isolated *via* preparative HPLC-2 with a retention time of 160 min and freeze dried to obtain a light yellow solid (LCMS yield: 35%, isolated yield, 0.02 g, 34%). [α]_D²⁰: -43.73°, (CHCl₃). ^1H NMR (500 MHz, CDCl₃): δ = 7.89 (1H, s, H3), 7.20 (1H, d, J = 8.42 Hz, H6), 7.83 (1H, d, J = 8.91 Hz, H7), 3.34 (2H, d, J = 7.02 Hz, H8), 5.26 (1H, m, H9), 1.78 (3H, s, H11), 1.75 (3H, s, H12), 2.34 (3H, s, H14), 7.63 (1H, d, J = 9.22 Hz, H5'), 6.90 (1H, d, J = 8.76 Hz, H6'), 2.29 (3H, s, H11''), 5.23 (2H, s, H1'''), 7.50 (2H, d, J = 8.64 Hz, H3'', H7''), 7.26 (2H, d, J = 8.21 Hz, H4'', H6''), 5.40 (1H, d, J = 8.63 Hz, H1'''), 5.36 (1H, t, J = 7.90 Hz, H2'''), 5.22 (1H, m,

H3'''), 5.13 (1H, t, $J = 8.77$ Hz, H4'''), 3.64 (1H, m, H5'''), 4.13 (1H, dd, $J = 5.69, 12.8$ Hz, H6a'''), 3.90 (1H, dd, $J = 2.13, 12.09$ Hz, H6b'''), 1.96–2.03 (12 H, s, $4 \times \text{CH}_3\text{CO}$). ^{13}C NMR (125 MHz, CDCl_3): $\delta = 166.50$ (C1), 130.84 (C2), 129.95 (C3), 134.77 (C4), 152.29 (C5), 122.98 (C6), 126.32 (C7), 28.93 (C8), 120.77 (C9), 134.12 (C10), 25.75 (C11), 18.00 (C12), 168.84 (C13), 20.89 (C14), 161.29 (C2'), 106.98 (C3'), 156.61 (C4'), 122.08 (C5'), 108.63 (C6'), 159.49 (C7'), 114.54 (C8'), 150.58 (C9'), 110.65 (C10'), 8.48 (C11'), 69.67 (C1''), 129.03 (C2''), 127.42 (C3''), 126.89 (C4''), 138.08 (C5''), 126.89 (C6''), 127.42 (C7''), 28.56 (C8''), 117.37 (C9''), 98.57 (C1'''), 71.15 (C2'''), 72.34 (C3'''), 67.86 (C4'''), 72.46 (C5'''), 61.34 (C6'''), 20.53–20.62 ($4 \times \text{CH}_3\text{CO}$), 170.34 (CH_3CO), 169.98 (CH_3CO), 169.52 (CH_3CO), 169.33 (CH_3CO). HMBC: H1''' linked with C4' and H1'' linked with C7'. ESI⁺ 1953 [2M + Na]⁺. HRMS: 966.2930, calculated for $\text{C}_{47}\text{H}_{47}\text{F}_3\text{N}_3\text{O}_{16}$: 966.2908.

4-Hydroxy-*N*-(8-methyl-2-oxo-7-((4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzyl)oxy)-4-(((3*R*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-2*H*-chromen-3-yl)-3-(3-methylbut-2-en-1-yl)benzamide (2) and 4-hydroxy-*N*-(4-hydroxy-8-methyl-2-oxo-7-((4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzyl)oxy)-2*H*-chromen-3-yl)-3-(3-methylbut-2-en-1-yl)benzamide (3)

Compound 15 (0.02 g, 0.02 mmol) was reacted with sodium methoxide (catalytic percentage) using the general acetate deprotection procedure (in CH_3OH) for 26 h. The product was purified by preparative HPLC-3 with a retention time of 201 min to afford compound 2 as a white powder (1.2 mg, 7.9%) and 256 min for compound 3 as a white powder (3 mg, 11%).

Compound 2: $[\alpha]_{\text{D}}^{20}$: -66.7° , (CHCl_3). ^1H NMR (500 MHz, MeOD_4): $\delta = 7.82$ (1H, s, H3), 6.87 (1H, d, $J = 8.63$ Hz, H6), 7.77 (1H, d, $J = 8.61$ Hz, H7), 3.42 (2H, m, H8), 5.38 (1H, m, H9), 1.77 (6H, s, H11, H12), 7.97 (1H, d, $J = 8.61$ Hz, H5'), 7.12 (1H, d, $J = 7.75$ Hz, H6'), 2.38 (3H, s, H11'), 5.33 (2H, s, H1''), 7.63 (2H, d, $J = 7.75$ Hz, H3'', H7''), 7.33 (2H, d, $J = 8.62$ Hz, H4'', H6''), 5.22 (1H, d, $J = 8.82$ Hz, H1'''), 3.49 (1H, m, H2'''), 3.30 (1H, m, H3'''), 3.36 (1H, m, H4'''), 3.12 (1H, m, H5'''), 3.68 (1H, d, $J = 11.8$ Hz, H6a'''), 3.52 (1H, m, H6b'''). ^{13}C NMR (125 MHz, MeOD_4): $\delta = 170.34$ (C1), 125.26 (C2), 130.98 (C3), 129.96 (C4), 160.66 (C5), 115.53 (C6), 128.52 (C7), 29.33 (C8), 123.41 (C9), 133.57 (C10), 26.1 (C11), 18.0 (C12), 168.84 (C13), 163.09 (C2'), 108.87 (C3'), 161.14 (C4'), 124.12 (C5'), 110.06 (C6'), 160.88 (C7'), 114.92 (C8'), 152.38 (C9'), 111.92 (C10'), 8.4 (C11'), 70.82 (C1''), 129.71 (C2''), 129.04 (C3''), 127.92 (C4''), 140.59 (C5''), 127.92 (C6''), 129.04 (C7''), 29.41 (C8''), 123.75 (C9''), 103.76 (C1'''), 75.35 (C2'''), 77.74 (C3'''), 70.82 (C4'''), 78.76 (C5'''), 61.96 (C6'''). HMBC: H1''' linked with C4' and H1'' linked with C7'. ESI⁻ 754 [M – H]⁻. HRMS: 756.2365, calculated for $\text{C}_{37}\text{H}_{37}\text{F}_3\text{N}_3\text{O}_{11}$: 756.2380.

Compound 3: ^1H NMR (500 MHz, MeOD_4): $\delta = 7.61$ (1H, s, H3), 6.78 (1H, d, $J = 8.99$ Hz, H6), 7.61 (1H, d, m, H7), 3.30 (2H, d, 7.64, H8), 5.17 (1H, s, H9), 1.68 (6H, s, H11, H12), 7.70 (1H, d, $J = 9.13$ Hz, H5'), 6.79 (1H, d, $J = 8.67$ Hz, H6'), 2.25 (3H, s, H11'), 5.07 (2H, s, H1''), 7.36 (2H, d, $J = 9.18$ Hz, H3''

H7''), 7.11 (2H, d, $J = 9.18$ Hz, H4'', H6''), 13.89 (1H, s, 4'-OH), 8.60 (1H, s, NH). ^{13}C NMR (125 MHz, MeOD_4): $\delta = 166.95$ (C1), 123.81 (C2), 129.92 (C3), 127.58 (C4), 158.97 (C5), 116.22 (C6), 127.64 (C7), 29.77 (C8), 120.59 (C9), 136.27 (C10), 25.82 (C11), 18.11 (C12), 161.76 (C2'), 103.06 (C3'), 153.40 (C4'), 122.57 (C5'), 108.67 (C6'), 159.12 (C7'), 114.15 (C8'), 149.81 (C9'), 111.07 (C10'), 8.50 (C11'), 69.91 (C1''), 129.02 (C2''), 127.41 (C3''), 126.85 (C4''), 138.29 (C5''), 126.85 (C6''), 127.41 (C7''), 28.70 (C8''), 122.10 (C9''). HMBC: H1'' linked with C7'. ESI⁻ 592 [M – H]⁻. HRMS: 594.1842, calculated for $\text{C}_{31}\text{H}_{27}\text{F}_3\text{N}_3\text{O}_6$: 594.1852.

4-Hydroxy-*N*-(8-methyl-4-oxo-2-((4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzyl)oxy)-7-(((2*S*,3*R*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-4*H*-chromen-3-yl)-3-(3-methylbut-2-en-1-yl)benzamide (4)

Compound 12 (0.01 g, 0.01 mmol) was reacted with sodium methoxide (catalytic percentage) using the general acetate deprotection procedure (in CH_3OH) for 22 h. The product was purified by preparative HPLC-3 with a retention time of 136 min to afford compound 4 as a white powder (1.2 mg, 7.9%). $[\alpha]_{\text{D}}^{20}$: -41.9° (ACN). ^1H NMR (500 MHz, MeOD_4): $\delta = 7.79$ (1H, s, H3), 6.87 (1H, d, $J = 6.4$ Hz, H6), 7.70 (1H, m, H7), 3.4 (2H, m, H8), 5.38 (1H, m, H9), 1.76 (6H, s, H11, H12), 7.97 (1H, d, $J = 8.61$ Hz, H5'), 6.85 (1H, d, $J = 6.3$ Hz, H6'), 2.39 (3H, s, H11'), 5.51 (2H, d, $J = 3.3$ Hz, H1''), 7.48 (2H, m, H3'', H7''), 7.23 (2H, d, $J = 7.23$ Hz, H4'', H6''), 5.06 (1H, d, $J = 7.9$ Hz, H1'''), 3.56 (1H, m, H2'''), 3.49 (1H, m, H3'''), 3.46 (1H, m, H4'''), 3.41 (1H, m, H5'''), 3.92 (1H, d, $J = 12.15$ Hz, H6a'''), 3.72 (1H, dd, $J = 5.5, 12.15$ Hz, H6b'''), 7.71 (1H, s, NH). ^{13}C NMR (125 MHz, MeOD_4): $\delta = 164.0$ (C1), 131.2 (C2), 131.0 (C3), 133.7 (C4), 148.6 (C5), 123.40 (C6), 128.47 (C7), 29.32 (C8), 123.39 (C9), 135.5 (C10), 26.00 (C11), 17.96 (C12), 163.81 (C2'), 105.9 (C3'), 164.2 (C4'), 127.99 (C5'), 115.53 (C6'), 160.53 (C7'), 116.3 (C8'), 154.4 (C9'), 112.9 (C10'), 8.63 (C11'), 75.20 (C1''), 130.4 (C2''), 129.33 (C3''), 112.97 (C4''), 139.8 (C5''), 112.97 (C6''), 129.33 (C7''), 28.19 (C8''), 126 (C9''), 102.21 (C1'''), 74.86 (C2'''), 78.17 (C3'''), 71.26 (C4'''), 78.39 (C5'''), 62.52 (C6'''). HMBC: H1''' linked with C7' and H1'' linked with C2'. ESI⁻ 755 [M – H]⁻. HRMS: 756.2365, calculated for $\text{C}_{37}\text{H}_{37}\text{F}_3\text{N}_3\text{O}_{11}$: 756.2380.

4-Hydroxy-*N*-(7-hydroxy-8-methyl-2-oxo-4-((4-(3-(trifluoromethyl)-3*H*-diazirin-3-yl)benzyl)oxy)-2*H*-chromen-3-yl)-3-(3-methylbut-2-en-1-yl)benzamide (5)

Compound 10 (0.01 g, 0.02 mmol) was reacted with sodium methoxide (catalytic percentage) using the general acetate deprotection procedure (in CH_3OH) for 15 h. The product was purified by preparative HPLC using 30–95% methanol–water in 180 min gradient elution to afford compound 5 as a white powder (6.6 mg, 50%). ^1H NMR (500 MHz, $\text{DMSO}-d_6$): $\delta = 7.63$ (1H, d, $J = 2.01$ Hz, H3), 6.83 (1H, d, $J = 8.01$ Hz, H6), 7.57 (1H, m, H7), 3.38 (2H, d, $J = 7.51$ Hz, H8), 5.31 (1H, m, H9), 1.80 (3H, s, H11), 1.70 (3H, s, H12), 2.42 (3H, s, H14), 7.57 (1H, m, H5'), 6.53 (1H, d, $J = 8.61$ Hz, H6'), 2.01 (3H, s, H11'), 3.44 (2H, m, H1''), 7.13 (2H, d, $J = 8.03$ Hz, H3'', H7''), 7.00 (2H, d, $J = 8.03$ Hz, H4'', H6''). ^{13}C NMR (125 MHz, $\text{DMSO}-d_6$): $\delta = 162.3$

(C1), 127.5 (C2), 130.0 (C3), 127.5 (C4), 158.4 (C5), 115.7 (C6), 127.4 (C7), 25.8 (C8), 120.9 (C9), 135.7 (C10), 29.5 (C11), 18.1 (C12), 169.90 (C13), 8.19 (C14), 188.9 (C2'), 67.4 (C3'), 168.0 (C4'), 127.5 (C5'), 112.1 (C6'), 162.1 (C7'), 112.3 (C8'), 153.4 (C9'), 126.0 (C10'), 8.0 (C11'), 43.3 (C1''), 129.2 (C2''), 130.5 (C3''), 126.5 (C4''), 132.8 (C5''), 126.5 (C6''), 130.5 (C7''), 43.0 (C8''), 120.6 (C9''). HMBC: 1'' linked with C4'. ESI⁺ 594 [M + H]⁺; HRMS: 594.1863, calculated for C₃₁H₂₇F₃N₃O₆; 594.1852.

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