Pergamon

Tetrahedron 56 (2000) 5649–5655

TETRAHEDRON

Highly Diastereoselective Synthesis of 4-*N*-Methylformamidino Trinem (GV129606), a Potent Antibacterial Agent

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Received 4 October 1999; accepted 21 December 1999

Abstract—In this paper a highly diastereoselective synthesis of 4-*N*-methylformamidino trinem **3** is reported. The route offers advantages compared to that previously used, i.e. the higher overall yield, the robustness, the avoidance of toxic reagents. Most of the compounds were isolated by precipitation, therefore reducing the number of chromatographic separations. The efficient conversion of 4-*N*-methylamino trinem **11** into GV129606 **3**, was obtained by a new methodology in which a scavenger resin was used. The route presented in this paper allowed the preparation of the material required for early development studies and demonstrates the versatility of cyclohexenyl azetidinone **12** in the synthesis of 4-substituted trinems.

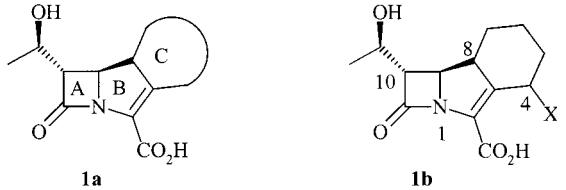
Introduction

The rapid emergence of bacteria with increased resistance to available drug therapies is becoming a serious worldwide health problem[1] that affects important classes of antibiotics including β-lactams, quinolones, macrolides, glycopeptides, aminoglycosides and tetracyclines.

Bacteria are exceedingly capable of evading antibacterials through processes of mutation and/or acquisition of resistance genes followed by selection and evolution in versatile ways to resist almost every new agent.[2] The major mechanisms of resistance to β-lactams involve the reduction in penetration through the outer membrane (for Gram-negative bacteria), the production of Penicillin Binding Proteins with a reduced affinity for inactivating agents, and the hydrolysis of the drug molecule by β-lactamases.[3]

Trinems[4] (Fig. 1), a class of β-lactam antibiotics discovered in our laboratories, are promising substances that can be effectively used to treat infections. The general structure of trinems **1a** is characterised by a tricyclic ring system in which ring C may be 5[5], 6 or 7[5e]-membered and may contain heteroatoms. When ring C is 6-membered as in structure **1b**, the position 4 is generally substituted and an hydroxyethyl side chain of defined absolute stereochemistry is present at position 10. Recently, some compounds having an alkylidene substituent on C-10 showing β-lactamase inhibitory activity[6] have also been published. Although several compounds with heteroatoms at various positions of ring C have been reported in the literature,[7] most of the work was directed towards the synthesis of compounds having a 6-membered carbocyclic system.[8] For this class we discovered that the stereochemistry of carbon atoms at position 4 and 8 is important in determining the biological profile of trinems. Generally the relative configuration 4α,8β represents the best compromise among the various properties required for a molecule to be effective as an antibacterial agent, e.g. activity, enzymatic and chemical stability, and pharmacokinetic profile.

Trinems are potent antibacterial agents, with a broad spectrum of activity against Gram positive and Gram negative strains, either aerobes or anaerobes, stable to most clinically relevant β-lactamases and to the human hydrolytic enzyme dehydropeptidase (DHP-I). In particular, Sanfetrinem[9] **2a** and its biolabile ester cilexetil[10] **2b** (see Fig. 2), are now undergoing phase II clinical studies, particularly for the treatment of infections caused by penicillin resistant strains.[11] As part of the research programme conducted in our laboratories we were interested in the introduction of a nitrogen containing functional group[12] at C-4. In particular we synthesized the 4-*N*-methylformamidino trinem **3** (see Fig. 2), which includes in its spectrum of activity the



1a **1b**

Figure 1.

Keywords: β-lactams; trinems; gram-negative bacteria; pseudomonads; diastereoselective synthesis.

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PII: S0040-4020(00)00414-2

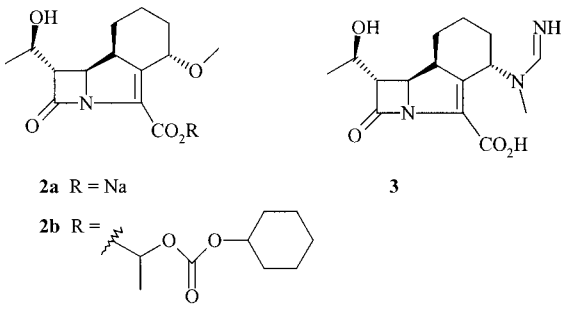


Figure 2.

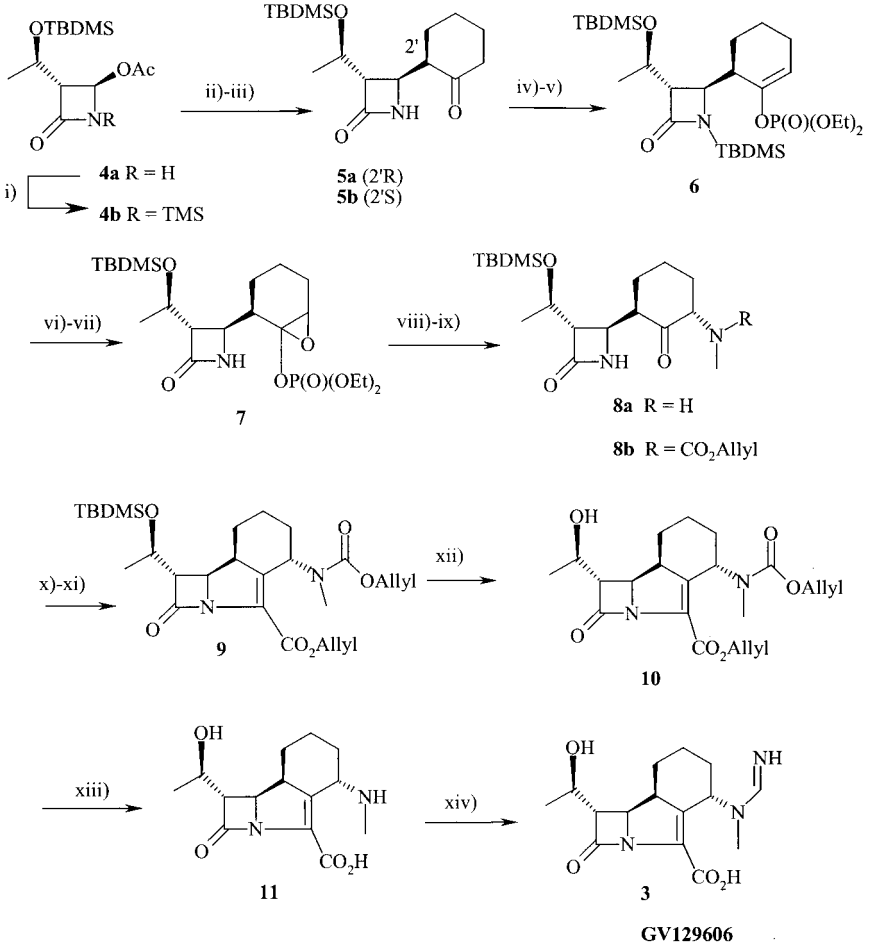
difficult strain *P. aeruginosa*, and has shown good efficacy and pharmacokinetic profile in animal models. The decision to better characterise the compound through preliminary pharmacological development studies prompted us to evaluate a more efficient synthesis, suitable for gram scale preparation.

Chemistry

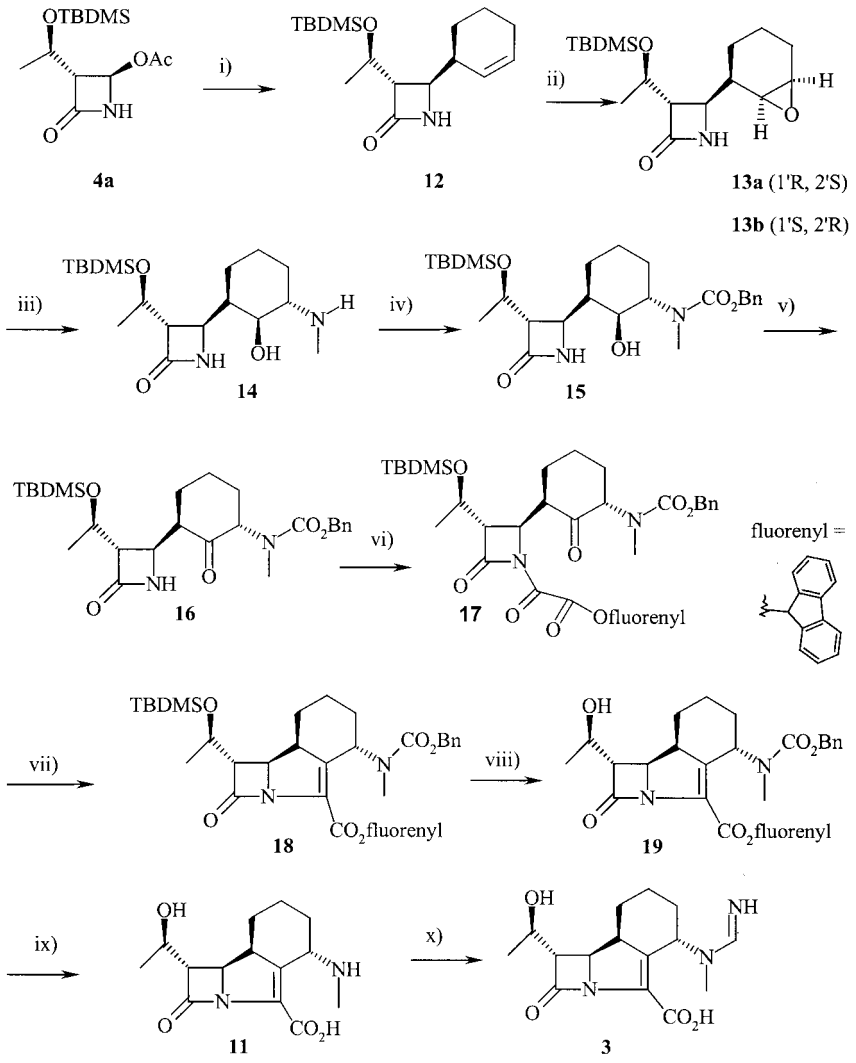
The original synthetic route[13] used to obtain GV129606 (**3**) required 14 steps starting from the commercially available 4-acetoxyazetidin-2-one **4a**[14] (Scheme 1), five chromatographic separations and a filtration through silica gel pad of the intermediates with an approximate overall yield of 0.5%.

The main drawbacks in this synthesis are:

(i) the moderate diastereoselectivity in favour of the desired isomer **5a** in the $SnCl_4$ catalysed addition of 1-(trimethylsilyloxy)cyclohexene to **4b** (**5a:5b**=4:1) resulting in the isolation of compound **5a** in 56% yield;[15]
(ii) the use of highly toxic diethylchlorophosphite in the preparation of compound **6**;
(iii) the instability of some intermediates like the epoxyphosphate **7** and the α-aminoketone derivative **8a** that caused variability in the recovered yield of carbamoyl derivative **8b**;
(iv) the lack of solid intermediates throughout the route that required extensive use of chromatographic separations;
(v) the complexity of the mixture obtained from the formimidoylation reaction of compound **11** and the consequent need for a reverse phase chromatography purification and lyophilisation to recover GV129606 **3**.



Scheme 1. Reagents and conditions: (i) TMSCl, TEA, CH_2Cl_2, 0°C; (ii) $SnCl_4$, 1-(trimethylsilyloxy)cyclohexane, −25°C, CH_3CN; (iii) SiO_2, MeOH, TEA, then crystallisation from petroleum; (iv) TBDMSCl, TEA, DMF, RT; (v) LHMDSA, $ClP(O)(Et)_2$, THF, −70°C; (vi) KF, MeOH, RT; (vii) MCPBA, CH_2Cl_2, 0°C; (viii) $MeNH_2$, K_2CO_3, ethyl acetate; (ix) $ClCO_2Allyl$, TEA, CH_2Cl_2, 0°C; (x) $ClCOCO_2Allyl$, TEA, CH_2Cl_2, RT (xi) $P(OEt)_3$, xylene, 120°C; (xii) TBAF, AcOH, THF, RT; (xiii) $Pd(Ph_3P)_4$, 5,5-dimethyl-1,3-cyclohexanedione; (xiv) Benzyloxyformimidate hydrochloride, pH=8 phosphate buffer, 0°C, then reverse phase chromatography on octadecylsilyl silica gel and lyophilisation.



Scheme 2. Reagents and conditions: (i) B-2-cyclohexenyl-(2-methylcyclohexyl)$_2$, Et_2Zn, 0°C; (ii) Magnesium monoperoxyphthalate, CH_2Cl_2, H_2O, 0°C; (iii) $MeNH_2$ (g), $LiClO_4$, CH_3CN, 60°C; (iv) $ClCO_2Bn$, TEA, CH_2Cl_2; (v) ClCOCOCl, DMSO, TEA, CH_2Cl_2; (vi) $ClCO_2$fluorenyl, TEA, toluene, RT; (vii) $P(OEt)_3$, 111°C; (viii) Bu_4NBr, KF, AcOH, THF; (ix) H_2 1 atm, 5% Pd/C, H_2O/isopropanol; (x) benzyloxyformimidate hydrochloride, amberlyst IRA68, H_2O/CH_3CN, then crystallization from H_2O/acetone.

To by-pass these issues, we planned for a new synthetic route (Scheme 2) in which one of the key intermediates, the cyclohexenyl azetidinone **12**, had been previously studied for the synthesis[16] of GV104326 **2a**. The greater diastereoselectivity in the condensation reaction of 4-acetoxy azetidin-2-one **4a** with B-2-cyclohexenyl-(2-methylcyclohexyl)$_2$ with respect to the trimethylsilylenol ether of cyclohexanone, the avoidance of the highly toxic diethylchlorophosphite and the overall reduction in the number of steps encouraged us to progress further with such a synthetic route.

The crystalline cyclohexenyl azetidinone **12**[17] was diastereoselectively oxidized under various reaction conditions to give the (1′*R*,2′*S*)-epoxide **13a** together with its (1′*S*,2′*R*) diastereoisomer **13b**. The use of *m*-chloroperoxybenzoic acid resulted only in a moderate diastereoselectivity **13a**/**13b**=85:15 but this could be improved to 95:5 with the use of the less reactive magnesium monoperoxyphthalate without significantly affecting the yield (>95%).

This 95:5 diastereomeric mixture was then treated with methylamine in acetonitrile at 60°C using lithium perchlorate as catalyst to give the corresponding aminoalcohol **14** that was isolated after precipitation from the reaction mixture as a single diastereoisomer in 78% yield.

At this point, the choice of suitable protecting groups that would allow isolation of crystalline intermediates to be removed simultaneously was of critical importance and required a certain number of optimisation studies. A significant improvement of the overall synthetic efficacy was achieved by the combination of a benzyloxycarbonyl group on the *N*-methyl amino moiety at C4 with a fluorenyl ester[18] at C2. Selective acylation of the secondary amino group with benzyl chloroformate gave the intermediate **15** in 86% yield, which was oxidised under Swern reaction conditions to give compound **16** as crystalline material (79%). The fluorenyloxalyl chloride, prepared from oxalyl chloride and fluorenyl alcohol, was reacted with **16** to form the oxalimido[19] intermediate **17** that was immediately

converted into the fully protected trinem derivative **18** in about 88% yield. Desilylation of **18** was initially performed with a large excess (14 equiv.) of tetrabutyl ammonium fluoride in THF either at room temperature or at 40°C. A convenient modification of the fluoride source was made by using a cheaper combination of tetrabutylammonium bromide and potassium fluoride. The hydroxyester intermediate **19**, obtained in 61% yield, was hydrogenated at atmospheric pressure using 5% palladium on charcoal. Optimisation of reaction conditions (see Experimental) allowed us to minimise the amount of residual starting material, the formation of side products side products deriving from the decomposition of reactive intermediates and reduction of the C2–C3 tetrasubstituted double bond. This resulted in an easier recovery and purification of the 4-*N*-methylamino trinem **11** by crystallisation from a mixture of water/isopropanol in 60% yield.

Still, the conversion of the 4-*N*-methylamino trinem **11** into the corresponding 4-*N*-methylformamidino trinem **3** represented a critical step due to the limited stability of both the starting material and the product to the reaction conditions used in the previous synthesis (Scheme 1). A similar formimidoylation reaction was also reported by Merck scientists for the synthesis of Imipenem.[20] Unfortunately, the procedures carefully optimised for the Imipenem synthesis were not efficient in our case and did not allow the isolation of the final GV129606 **3** with the required purity. The main problem associated with this procedure was the contamination of the product **3** with both inorganic salts and by-products deriving from the decomposition of the trinem derivatives.

It was therefore attempted to perform the reaction in organic solvents using highly lipophilic tertiary amines, which could trap the hydrochloric acid from the benzyloxy formimidate forming an ammonium salt that could be removed from the aqueous solution. Satisfactory results were obtained with *N*,*N*-dimethyldodecylamine, that gave the desired compound **3**, in good yield. The main drawback of this protocol was the high number of extractions (>20) required for a complete removal of the ammonium salt from the aqueous solution. The use of amines having more lipophilic chains was unsuccessful as we observed the formation of emulsions that were hardly treatable. Therefore, we switched our attention towards basic resins as proton scavenger, suitable for use in aqueous solutions and easily removable by filtration. The study has been extended to several commercially available amine based resins covering a range of basicity. Best results were obtained by using tertiary amine resin IRA68®. This allowed us to obtain a yield >90% in solution by carrying out the reaction in a mixture of water and acetonitrile at 0°C. Removal of the organic solvent under reduced pressure and addition of acetone at 0°C allowed crystallisation of GV129606 **3** with a purity >95 and 42% yield.

Conclusions

A new, highly diastereoselective synthetic route to GV129606 **3** was established using the cyclohexenyl azetidinone **12** as key intermediate. This represents a further example of the synthetic utility of compound **12** in the synthesis of 4-substituted trinems.

The route described in Scheme 2 avoids the use of the highly toxic diethylchlorophosphite and the formation of unstable intermediates, such as epoxyphosphate **7** and α-amino ketone **8a**.

The appropriate choice of protecting groups for both the amino and carboxylic functions, allowed the elimination of several chromatographic steps with respect to the original route and the efficient simultaneous removal of both protecting groups by heterogeneous catalysis. In addition, the use of the tertiary amine resin IRA68® as proton scavenger in the last step of the synthesis greatly simplified the work-up improving the amount and quality of the recovered final compound.

The number of steps was reduced from 14 to 10 and the number of purifications by silica gel chromatography was limited to 2 instead of the 5 previously required. This allowed us to recover GV129606 **3** in about 7% overall yield with respect to the 0.5% of the original route. The synthesis described in Scheme 2 was practical enough to allow the isolation of the 4-*N*-methylformamidino trinem **3** in gram quantities and with the required purity to support early development studies.

Further studies aimed at finding a more efficient route to GV129606 **3** are required for larger scale production of this compound.

Experimental

Materials and apparatus

The IR spectra were collected on a Bruker IFS48 instrument using a dispersion in nujol on KBr pellets, or in solution of $CDCl_3$. The NMR spectra were recorded using a UMX 300 Varian (300 MHz) instrument or an AC 200 Bruker (200 MHz) instrument. The MS spectra were recorded with a VG quattro Fison instrument using a FAB+ionization technique (70 eV). Melting points were performed using a Buchi 530 and are uncorrected. The HPLC analysis were executed on Jasco PU 980 instrument equipped with a Jasco UV 975 Detector using a Hypersil BDS C18 reverse phase column (20×0.46 cm) at room temperature. Compounds were purified by flash chromatography using Merck silica gel 60 (230–400 mesh). The reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60 F254 TLC plates. Reagents and solvents (sure seal package) used in this work were purchased from Aldrich, Fluka and Janssen and used directly without any purification.

Methods

Compound 13. Cyclohexenyl azetidinone **12**[17] (50 g, 162 mmol) was dissolved in dichloromethane (550 ml) at 0°C, water (500 ml) and magnesium monoperoxyphthalate hexahydrate (112 g, 226 mmol) were added and the mixture vigorously stirred for 24 h. A solution of 2 M sodium

carbonate (600 ml) was added and the two phase system was stirred for 20 min. The two phases were separated and the aqueous solution was extracted with dichloromethane (650 ml), the organics were combined and washed with a 10% solution of sodium metabisulphite (500 ml). The organic solution was dried over sodium sulphate and the solvent removed under reduced pressure to give a white solid (51.5 g, 97.8%). **13a** ^{1}H NMR (300 MHz, δ=ppm; $Si(CH_3)_4$ in $CDCl_3$): 5.97 (br.s, 1H), 4.22 (m, 1H), 3.77 (dd, 1H), 3.16 (t, 1H), 3.12 (m, 1H), 3.01 (m, 1H), 2.00 (m, 1H), 1.96 (m, 1H), 1.86 (m, 1H), 1.55 (m, 1H), 1.40 (m, 1H), 1.26 (d, 3H), 1.22 (m, 2H), 0.87 (s, 9H), 0.07 (s, 6H). IR (ν_{max}/cm^{-1}): 3413 (NH, str.), 1757 (C=O str.). MS (*m*/*z*): 326 (MH^+). White solid, melting point 134–136°C. $[\alpha]_D$=35.1° c=0.61 in CH_2Cl_2. **13b** ^{1}H NMR (300 MHz, δ=ppm; $Si(CH_3)_4$ in $CDCl_3$): 6.00 (br.s, 1H), 4.13 (m, 1H), 3.55 (dd, 1H), 3.16 (m, 1H), 3.08 (m, 1H), 2.93 (dd, 1H), 2.10 (m, 1H), 1.99 (m, 1H), 1.69 (m, 1H), 1.62 (m, 1H), 1.43 (m, 2H), 1.27 (d, 3H), 0.90 (m, 1H), 0.88 (s, 9H), 0.09 (s, 6H). IR (ν_{max}/cm^{-1}): 3414 (NH, str.), 1757 (C=O str.). MS (*m*/*z*): 326 (MH^+). White solid, melting point 96–98°C. $[\alpha]_D$=−20° c=0.59 in CH_2Cl_2.

Compound 14. Compound **13a** (51 g, 156 mmol) was placed in a three necks round bottom flask equipped with an acetone dry ice trap, dissolved in dry acetonitrile (500 ml), then anhydrous lithium perchlorate (100 g, 940 mmol) was added and the mixture was heated at 60°C to obtain a clear solution. Methylamine was bubbled for 30 min and the solution was stirred for 3.5 h at the same temperature. The solution was cooled at room temperature and the volume reduced to 150 ml, poured into water (1 l) and extracted with diethyl ether (3×500 ml). The organic solution was dried over sodium sulphate and the solvent removed under reduced pressure to afford 65 g of a crude that was treated with cyclohexane to give the methylamino derivative **14** (43.5 g, 78%). ^{1}H NMR (300 MHz, δ=ppm; $Si(CH_3)_4$ in $CDCl_3$): 6.26 (s, 1H), 4.20 (m, 1H), 3.80 (m, 1H), 3.72 (dd, 1H), 3.13 (m, 1H), 2.67 (m, 1H), 2.49 (s, 3H), 2.02 (m, 2H), 1.70–1.12 (m, 5H), 1.31 (d, 3H), 0.91 (s, 9H), 0.12 (s, 6H). IR (CDCl3): 3416 (NH, OH str.), 1753 (C=O str.). MS (*m*/*z*): 357 (MH^+). White solid.

Compound 15. Compound **14** (71.5 g, 200 mmol) was dissolved in dichloromethane (400 ml) under a nitrogen atmosphere at 0°C, diisopropyl ethylamine (63 ml, 360 mmol) and benzylchloroformate (43 ml, 300 mmol) were sequentially added and the reaction stirred for 2 h. The reaction mixture was poured into a saturated solution of ammonium chloride (2 l) and the organic phase separated. The aqueous solution was extracted with dichloromethane and the organics were joined and washed with a 2% solution of sodium bicarbonate (3×600 ml). The solution was dried over sodium sulphate and the solvent removed under reduced pressure to afford a crude product (120 g) that treated with cyclohexane gave compound **15** (84 g, 86%). ^{1}H NMR (300 MHz, δ=ppm; $Si(CH_3)_4$ in $CDCl_3$): 7.40–7.20 (m, 5H), 6.02 (s, 1H), 5.20–5.00 (m, 2H), 4.21 (m, 1H), 4.10–4.00 (m, 1H), 3.84 (dd, 1H), 3.88–3.76 (m, 1H), 3.16 (m, 1H), 2.88 (s, 3H), 3.00–2.70 (m, 1H), 2.26 (m, 1H), 1.92–1.82 (m, 1H), 1.76–1.68 (m, 1H), 1.58 (m, 1H), 1.55–1.35 (m, 3H), 1.31 (d, 3H), 0.88 (s, 9H), 0.08 (s, 6H). IR ($CDCl_3$): 3406(OH str.), 3150 (NH str.), 1755 (C=O str.), 1691 (C=O str.). MS (*m*/*z*): 491 (MH^+). White solid.

Compound 16. Oxalyl chloride (19.4 ml, 222 mmol) was dissolved in dry dichloromethane (200 ml) under nitrogen atmosphere and cooled at −70°C. A solution of dimethyl sulphoxide (31.6 ml, 444 mmol) in dichloromethane (50 ml) was added dropwise maintaining the temperature below −60°C and the mixture stirred for further 20 min. A solution of intermediate **15** (54.5 g, 111 mmol) in dichloromethane (500 ml) was slowly added at −70°C and allowed to react for 1 h. Triethylamine (124 ml, 888 mmol) was added and the temperature was raised to 10°C, a saturated solution of ammonium chloride (1 l) was added and extracted with dichloromethane. The organic phase was washed with a 2% solution of sodium bicarbonate (600 ml), water (2×600 ml), and dried over sodium sulphate. The solvent was removed under reduced pressure to give a solid material (66 g) which was redissolved in the minimum amount of ethyl acetate and the ketoazetidinone **16** was crystallized by addition of cyclohexane (43 g, 79%). ^{1}H NMR (300 MHz, δ=ppm; $Si(CH_3)_4$ in $CDCl_3$): 7.34–7.30 (m, 5H), 5.99 (s, 1H), 5.11 (s, 2H), 4.56 (m, 1H), 4.19 (dd, 1H), 4.04 (m, 1H), 2.99 (dd, 1H), 2.87 (s, 3H), 2.66 (m, 1H), 2.20–1.80 (m, 6H), 1.10 (d, 3H), 0.87 (s, 9H), 0.065 (s, 6H). IR ($CDCl_3$): 3490 (NH str.), 1763 (C=O str.), 1718 (C=O str.), 1691 (C=O str). MS (*m*/*z*): 489 (MH^+). White solid, melting point 147–148°C.

Fluorenyloxalyl chloride. 9-Hydroxyfluorene (8.4 g, 46.1 mmol) was dissolved in THF (60 ml) and added dropwise over 30 min to a solution of oxalyl chloride (7.6 ml, 92.2 mmol) in THF (40 ml) at 0°C under nitrogen atmosphere. The reaction mixture was stirred for 1 h, the solvent and excess oxalyl chloride were removed under reduced pressure to give a pale green solid that was readily used in the following reaction. ^{1}H NMR (300 MHz, δ=ppm; $Si(CH_3)_4$ in $CDCl_3$): 7.68 (d, 2H), 7.57 (d, 2H), 7.46 (dt, 2H), 7.32 (dt, 2H), 6.84 (s, 1H). IR ($CDCl_3$): 1778 (C=O str.), 1749 (C=O str.). MS (*m*/*z*): 274–272 (M^+) EI.

Compound 17. Intermediate **16** (10 g, 20.5 mmol) was dissolved in dichloromethane (20 ml), anhydrous potassium carbonate (5.6 g, 41 mmol) and freshly distilled pyridine (6.6 ml, 82 mmol) were added and the resulting suspension stirred at 0°C. A freshly prepared solution of fluorenyloxalyl chloride (see above) in dichloromethane (60 ml) was added through a dropping funnel equipped with a sintered glass to remove the fluorenyl oxalic acid. After the addition was complete, the temperature was allowed to rise to 25°C and the reaction monitored by thin layer chromatography. After disappearance of compound **8c**, the solvent was reduced to 1/3 of initial volume under reduced pressure. The slurry so obtained was triturated with diethyl ether and filtered over a silica gel pad, washing several times with diethyl ether. The organic solution was extracted with a saturated solution of sodium bicarbonate, with a solution of 0.4 M HCl and brine. The organic solution was dried over anhydrous sodium sulphate and the solvent removed under reduced pressure to give 14 g of the oxalimido intermediate **17** (94.6% yield). ^{1}H NMR (300 MHz, δ=ppm; $Si(CH_3)_4$ in CDCl3): 7.66 (m, 4H), 7.5–7.2 (m, 9H), 6.97 (s, 1H), 5.09 (s, 2H), 4.65 (dd, 1H), 4.50 (t, 1H), 4.27 (m, 1H), 3.21 (m, 1H), 2.86 (s, 3H),

2.84 (m, 1H), 2.20–1.70 (m, 6H), 1.09 (bs, 3H), 0.75 (s, 9H), 0.02 to (−0.08) (ss, 6H). IR ($CDCl_3$): 1807 (C═O str.), 1732 (C═O str.), 1699 (C═O str.). MS (*m/z*): 725 (MH^+). White solid, melting point 50–60°C.

Compound 18. The oxalimido derivative **17** (8.9 g, 12.3 mmol) was dissolved in nonane (178 ml) at 50°C and triethyl phosphite (8.4 ml, 49.2 mmol) was added. The resulting solution was heated at reflux for 5 h, cooled at room temperature and treated with a 5% solution of hydrogen peroxide (104 ml). The mixture was stirred for 2 h and extracted with ethyl acetate. The organic phase was extracted twice with water, dried over sodium sulphate and concentrated under reduced pressure. The crude product was purified with a silica gel pad using a mixture of cyclohexane and ethyl acetate in a 3/1 ratio as eluant. After removal of the solvent, compound **18** was obtained (7.3 g, 88.5% yield). ^1H NMR (300 MHz, δ=ppm; $Si(CH_3)_4$ in $CDCl_3$): 7.7–7.6 (m, 4H), 7.4–7.2 (m, 9H), 6.86 (s, 1H), 5.32 (t, 1H), 4.98 (dd, 2H), 4.16 (m, 1H), 4.07 (dd, 1H), 3.21 (dd, 1H), 3.17 (m, 1H), 2.94 (s, 3H), 2.11 (m, 1H), 1.85 (m, 1H), 1.7–1.3 (m, 4H), 1.20 (d, 3H), 0.82 (s, 9H), 0.04–0.01 (ss, 6H). IR ($CDCl_3$): 1782 (C═O str.), 1711 (C═O str.). MS (*m/z*): 725 (MH^+). White solid, melting point 50–55°C.

Compound 19. Compound **18** (11.5 g, 16.6 mmol) was dissolved in THF (57.5 ml) and acetic acid (6.64 ml, 116.2 mmol), tetrabutylammonium bromide (26.2 g, 81.34 mmol), cesium fluoride (12.4 g, 81.34 mmol) were sequentially added. The reaction mixture was warmed to 40°C, water (0.732 ml, 40.67 mmol) was added and stirring was continued for 4 h. After cooling at room temperature, the reaction was diluted with ethyl acetate and washed with an ice cold solution of saturated sodium bicarbonate, saturated ammonium chloride and brine. The organic solution was dried over sodium sulphate and the solvent removed under reduced pressure. The crude product was purified by flash chromatography, eluting with a mixture of ethyl acetate and cyclohexane in a 3:1 ratio to give compound **19** as a white solid (5.86 g, 61% yield). ^1H NMR (300 MHz, δ=ppm; $Si(CH_3)_4$ in $CDCl_3$): 7.63 (d, 4H), 7.4–7.2 (m, 9H), 6.89 (s, 1H), 5.29 (t, 1H), 5.00 (m, 2H), 4.17 (m, 1H), 4.05 (dd, 1H), 3.22 (dd, 1H), 3.17 (m, 1H), 2.92 (s, 3H), 2.12 (m, 1H), 1.90 (m, 1H), 1.75–1.40 (m, 4H), 1.28 (d, 3H). IR ($CDCl_3$): 3420 (OH str.), 1776 (C═O str.), 1695 (C═O str.). MS (*m/z*): 579 (MH^+). White solid, melting point 77–80°C.

Compound 11. Compound **19** (3.15 g, 5.4 mmol), dissolved in isopropanol (90 ml) and water (90 ml), was slowly added under vigorous stirring. The solution was flushed with nitrogen and Pd/C 20%, w/w (0.63 g) was added. The resulting suspension was hydrogenated at 1 atm for 2.5 h, filtered through a Celite pad and extracted twice with diethyl ether. The aqueous solution was concentrated under reduced pressure to a small volume until a solid material started to precipitate. Isopropanol was added dropwise with stirring and a white precipitate was filtered (0.88 g, 60% yield). ^1H NMR (300 MHz, δ=ppm; $Si(CH_3)_4$ in D_2O): 4.81 (m, 1H), 4.13 (m, 1H), 4.10 (dd, 1H), 3.38 (dd, 1H), 3.04 (m, 1H), 2.48 (s, 3H), 1.97 (m, 1H), 1.84–1.20 (m, 5H), 1.12 (d, 3H), 1. IR ($CDCl_3$): 3462–3412 (NH, OH str.), 1755 (C═O str.). MS (*m/z*): 281 (MH^+). White solid.

Compound 3. Compound **11** (2 g, 7.14 mmol) was dissolved in water (50 ml), acetonitrile (50 ml) was added and the resulting solution was cooled at 0°C. A suspension of freshly washed IRA68® resin (66.7 ml, 105 mmol) in water/acetonitrile 1:1, was added, then benzyloxyformimidate hydrochloride (4.3 g, 25 mmol) was slowly added over 1 h. The reaction was monitored by HPLC and the resin removed by filtration through filter paper, washed with water (10 ml) and the collected fractions were washed with diethyl ether. The aqueous layer was concentrated under reduced pressure, cooled at 0°C and acetone was slowly added with stirring until a precipitate of GV129606 **3** was obtained. The solid was then filtered through paper and washed with ice cold acetone to give 920 mg of final compound (42%). ^1H NMR (300 MHz, δ=ppm; $Si(CH_3)_4$ in D_2O): 7.72 (s, 1H), 5.13 (t, 1H), 4.10 (m, 1H), 4.04 (dd, 1H), 3.30 (dd, 1H), 2.88 (m, 1H), 2.85 (s, 3H), 2.17 (m, 1H), 1.80 (m, 4H), 1.35 (m, 1H), 1.07 (d, 3H). IR ($CDCl_3$): 3420–3240 (NH, OH str.), 1745 (C═O str.), 1715 (C═O str.), 1670 (C═N str.). MS (*m/z*): 308 (MH^+). White solid.

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