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Design and synthesis of new bicyclic diketopiperazines as scaffolds for receptor probes of structurally diverse functionality†

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Abstract

Diketopiperazines (DKPs) are a common motif in various biologically active natural products, and hence they may be useful scaffolds for the rational design of receptor probes and therapeutic agents. We constructed a new bicyclic scaffold that combines a DKP bridged with a 10-membered ring. In this way we obtained a three-dimensional molecular skeleton, with several amendable sites that provide a starting point to design a new combinatorial library having diverse substituent groups. Structural variation is based upon the flexibility of alkylation of the nitrogen atoms of the DKP and on the side-chain olefin. We obtained a 10-membered secondary ring through a ring-closure metathesis reaction using the second generation Grubbs catalyst. Rings containing both O-ethers and S-ethers were compared. *N*-Alkyl or arylalkyl groups were introduced optionally at the two Nα-atoms. This is a general scheme that will allow us to test rings of varying sizes, linkages, and stereochemical parameters. The DKP derivatives were tested for activity in astrocytoma cells expressing receptors coupled to phospholipase C. Inhibitory effects were observed for signaling elicited by activation of human nucleotide P2Y receptors but not m3 muscarinic receptors. Compound **20** selectively inhibited calcium mobilization (IC_{50} value of 486 ± 16 nM) and phosphoinositide turnover elicited by a selective $P2Y_1$ receptor agonist, but this compound did not compete for binding of a radiolabeled nucleotide-competitive receptor antagonist. Therefore, the new class of DKP derivatives shows utility as pharmacological tools for P2Y receptors.

Introduction

The diketopiperazine (DKP) ring has been noted as a motif in various biologically active natural products. Examples include the commercial antibiotic bicyclomycin,[1] the fungal metabolites TAN-14 A, C and E,[2] and the thaxtomin A and B.[3] DKPs may be useful scaffolds for the rational design of receptor probes and drugs. Owing to the presence of multiple H-bond acceptors and donors and the side chains of the component amino acids, DKPs have multiple sites for structural elaboration of defined stereochemistry. Others have

†Electronic supplementary information (ESI) available: coordinates of two representative conformations, and molecular model of compound **20** (in PDB format). See http://www.rsc.org/suppdata/ob/b4/b416349d/

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explored the synthesis of DKPs of varied structure,[4–8] and combinatorial/polymer-supported syntheses of DKPs have been reported.[9–12]

One of the limitations of conventional medicinal chemical agents, as applied to the new domain of chemical genetics,[13,14] is the typical planarity found in most known pharmaceutical substances discovered through organic synthesis. We desired to explore a three-dimensional molecular skeleton, which could be regarded as a starting point to design a new combinatorial library having diverse substituent groups. In the present study we have built a new bicyclic scaffold that combines a DKP additionally bridged with a 10-membered ring. A similar approach to synthesize a series of bridged DKPs, which were desired as mimics of a peptide β-turn found in peptide hormones, was introduced by Reitz and co-workers.[15]

Construction of the DKP skeleton was achieved with amino acid derivatives *N*-Boc-L-serine and L-allylglycine, thus providing great flexibility for structural variation, including substitution at the nitrogen atoms of the DKP and on the side-chain olefin. The 10-membered ring was obtained through a ring-closure metathesis (RCM) reaction using the second generation Grubbs catalyst.[16,17] This methodology provides a bicyclic DKP template with ether and olefinic groups present. This is a general scheme that will allow us to test larger and smaller rings, vary the ether linkage and manipulate stereochemical parameters. The ether and olefinic functionality is desired for both chemical advantages (*i.e.* the ability to derivatize at these positions) and biological advantages (*i.e.* to provide additional recognition elements for interaction with receptors and other biopolymers).

Selected bicyclic DKP analogues were subjected to biological assays in a variety of receptor-based systems. Several of the derivatives were found to inhibit the activity of extracellular nucleotides through G protein-coupled P2Y receptors and subsequent biochemical steps. Eight mammalian subtypes of [18–21] such receptors ($P2Y_{1,2,4,6,11–14}$) are currently sequence-defined. The P2Y receptors are present in the cardiovascular, immune, and nervous systems, as well as in other systems. They are currently the focus of intense drug discovery efforts to design selective agonists and antagonists, which are lacking for most of the subtypes. There are numerous therapeutic possibilities under exploration for P2Y receptor modulators. For example, antagonists of the $P2Y_1$ and $P2Y_{12}$ receptors are of interest as antithrombotic agents.[22–32] Agonists of the $P2Y_2$ receptor are of interest in the treatment of pulmonary diseases, including cystic fibrosis. The $P2Y_6$ receptor has recently been implicated in protection against apoptosis induced by TNFα.[33]

Results

Chemical synthesis

A new bicyclic scaffold that combines a DKP bridged with a 10-membered ring was prepared. This 10-membered secondary ring was obtained through a RCM reaction using the second generation Grubbs catalyst. The starting materials in this synthesis (Schemes 1–3) were two amino acid derivatives of defined stereochemistry, *N*-Boc-L-serine and L-allylglycine, which provided the bicyclic DKP template with both ether and olefinic groups present. In order to substitute the O-ether moiety with an S-ether, L-cysteine **6** was used as the starting material instead of *N*-Boc-L-serine **1**.

The first step of the synthesis was the introduction of the allyl group in the *N*-Boc-L-serine and L-cysteine using the methods reported in the literature.[34,35] Esterification of compound **2** with $TMSCHN_2$ followed by removal of the Boc group with TFA afforded the amino ester **5**. The intermediate **8** was obtained after esterification of the amino acid **7** (Scheme 1).

The amino methyl ester **5** was subjected to reductive amination in the presence of 2,4-dimethoxybenzaldehyde or 2,4,6-trimethoxybenzaldehyde to give compounds **13** and **14**, respectively. Following the same procedure, the thio derivatives **15** and **16** were obtained from **8** (Scheme 1). Our initial procedure, in order to minimize racemization, involved mixing the amine, $NaBH(OAc)_3$ and the aldehyde simultaneously in CH_2Cl_2. [15,36,37] The reactions carried at room temperature for 2 h afforded the desired compounds but with racemization that was partial (70: 30) for the dimethoxybenzyl derivatives **13** and **15** and total (50: 50) for the trimethoxybenzyl derivatives **14** and **16**. An alternative route consisted of the reductive amination followed by esterification. The amino acid derivative **4** was subjected to reductive amination using $NaBH_3CN$ in methanol at room temperature for 24 h to obtain **9**, which after esterification with $TMSCHN_2$ provided **13** with less than 5% of racemization. Following the same procedure, **14**–**16** were obtained from **4** or **7** with a similar degree of racemization.

Fmoc-L-allylglycine (**17**) was coupled with the secondary amine **13** using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (HATU), 1-hydroxy-7-azabenzo-triazole (HOAt) and *N*-ethylmorpholine (NEM) in a mixture of CH_2Cl_2–DMF (9: 1) to obtain the dipeptide **18** (Scheme 2). RCM of **18** (1 mM) using the second generation Grubbs catalyst in dichloromethane at room temperature provided **19**. When the first generation Grubbs catalyst was used with **18** no reaction was observed. Finally, removal of the Fmoc group of **19** using a mixture of piperidine–CH_2Cl_2 (1: 4) was accompanied by *in situ* formation of the DKP, and compound **20** was obtained in high yield (93%).

Similar reaction sequences starting from compounds **14**, **15** and **16**, instead of **13**, provided the bicyclic DKP templates **25**, **33** and **36**.

In order to introduce more diversity in the scaffold, the amide in the DKP was alkylated with methyl and benzyl groups.[38] Compounds **20** and **25** were treated with NaH and the resulting anions reacted with methyl iodide and benzyl bromide to give **21**, **22**, **27** and **28**.

The trimethoxybenzyl group in **25**, **27** and **28** was removed easily under acidic conditions; however, the normally acid-labile dimethoxybenzyl group in **20** was stable under these conditions.[36]

By this route the new bicyclic scaffold was obtained through RCM followed by the formation of the DKP ring (Scheme 2). The possibility was explored to obtain these derivatives through a new route, by which the DKP ring was built first and then the 10-membered ring was formed (Scheme 3). In this case, compound **18** reacted with the mixture of piperidine–CH_2Cl_2 (1: 4) to obtain the DKP **37**. The RCM reaction of **37** using first generation Grubbs catalyst was not satisfactory. When the second generation catalyst was used and the reaction was performed in conditions of high dilution (0.2 mM), **20** (21%) was obtained with the dimer **38** (43%). However, with higher concentrations (40 mM) only the dimer **38** was obtained (79%).

Pharmacological activity

Compounds **20**–**22**, **25**–**30**, **33**, and **36** were screened at a 10 lM concentration in an assay of phospholipase C in 1321N1 human astrocytoma cells stably expressing the human $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors. The biological characterization of the dimeric **38** will be reported elsewhere. In $P2Y_1$ receptor-expressing cells, inhibition of agonist (30 nM 2-MeSADP)-induced phospholipase C (PLC) was observed only for **20** (45 ± 10%), **33** (33 ± 4%), and **36** (18 ± 4%). At the other P2Y receptor subtypes, substantial inhibition of PLC activity (*ca.* 50% at 10 μM) was observed only for compounds **21** and **36** at the $P2Y_4$

receptor and compound **20** at the $P2Y_6$ receptors. Compound **20** and other DKPs (Fig. 1) inhibited the mobilization of intracellular $[Ca^{2+}]$ in astrocytoma cells stably expressing the human $P2Y_1$ receptor, with concentration-dependent reduction of the maximal effect. Compound **20** was the most potent with complete inhibition of $[Ca^{2+}]$ mobilization at 10 μM. Increasing concentrations of compound **20** progressively attenuated the 2MeSADP effect (Fig. 2). Compound **20** was found to inhibit $[Ca^{2+}]$i transients elicited by 2MeSADP with an IC_{50} value of 486 ± 16 nM. Control experiments in which PLC was stimulated *via* an endogenous m3 muscarinic receptor (using carbachol) in the control astrocytoma cells or *via* heterologously expressed $P2Y_{2,4,6}$ receptors (using the appropriate nucleotide) demonstrated insignificant or weak inhibition by compound **20**. Thus, the inhibitory effects of **20**, albeit insurmountable, were selective for signaling induced by $P2Y_1$ receptor activation. However, in a radioreceptor binding assay compound **20** at 10 μM failed to compete for the binding of a specific $P2Y_1$ antagonist radioligand, [^{3}H]MRS2279.[39] Therefore, the binding of the DKP was not occurring at the principal nucleotide binding site of this receptor.

As an indication of the selectivity of these derivatives for the P2Y receptor pathway, interactions with a limited number of other receptors were examined (data not shown). Binding experiments at the human A_1 AR expressed in CHO cell membranes[40] indicated that compound **20** at 10 μM displayed no significant inhibition of radioligand binding. Similarly, this compound failed to displace binding at the mouse TRH receptors, or to either activate or antagonize functional effects of the m3 muscarinic receptor or the human calcium sensing receptor.

By means of a molecular dynamics simulation we carried out a conformational analysis of compound **20**. The DKP ring-puckering coordinates ($\Theta \cong -80°$; $P_2 \cong -30°$; $Q \cong 40°$) indicated a boat–screw/boat conformation.

Two different stable conformations were found for the 10-membered ring: in the first conformation the ring bends toward the dimethoxybenzyl group, while in the second one it bends in the opposite direction. Two diametrically opposite stable conformations were also found for the dimethoxybenzyl group. The coordinates of two representative conformations, in PDB format, are supplied as ESI.[†]

Consistent with the non-competitive nature of these ligands, the stable conformations of compound **20** do not resemble the bound conformation of the competitive $P2Y_1$ antagonist MRS2279, neither by steric nor electronic criteria.[41]

Discussion

These DKP scaffold molecules were intended for broad screening with no *a priori* bias for any particular protein target. Among the targets thus far examined, inhibition was associated exclusively with the P2Y receptors. These receptors are diverse in the nature of their endogenous nucleotide ligands. Adenine nucleotides are required for activation of the $P2Y_1$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$ subtypes, and uracil nucleotides activate $P2Y_2$, $P2Y_4$, and $P2Y_6$ subtypes. The $P2Y_2$ receptor is equipotently activated by UTP and ATP. The most recently identified subtypes are the $P2Y_{13}$ receptor, which is present in human monocytes and lymphocytes,[42] and the $P2Y_{14}$ receptor, which is activated by UDP-glucose.[43] P2Y receptor ligands are being investigated for therapeutic applications in the cardiovascular, endocrine, and other systems. Exploration of selective agonists and antagonists modulators is most advanced at the $P2Y_1$ and $P2Y_{12}$ receptors, but at most of the P2Y receptors selective pharmacological probes are lacking.[22–32] Compound **20** was shown to antagonize signaling of the $P2Y_1$ receptor selectively and with moderate potency. At the $P2Y_1$ receptor, the

previously described high affinity antagonists are nucleotide derivatives and therefore highly charged, which is highly limiting in pharmacological studies due to low bioavailability and stability.

Therefore, owing to the ability to modify the chemical functionality of this new class of DKP derivatives and the already demonstrated biological activity, it shows promise as an approach to designing new pharmacological tools. Further study will be required to explore the mechanism of inhibition of P2Y receptor-induced effects and to determine the overall pharmacological selectivity of this class of compounds. It must still be determined if manipulation of functional groups on the DKP derivatives is able to vary the spectrum of interaction of these scaffold molecules with signaling of specific P2Y receptor subtypes, and among varied biochemical target proteins in general. If the inhibition by the DKP is found to occur in direct association with P2Y receptors, *e.g.* at an allosteric site, several of the compounds already prepared, such as compound **20**, will provide insurmountable antagonists with receptor selectivity.

Experimental

Chemical synthesis

Reagents and solvents were purchased from Sigma-Aldrich (St. Louis, MO). Compounds **2** and **7** were synthesized as reported.[34,35]

^{1}H NMR spectra were obtained with a Varian Gemini 300 spectrometer using $CDCl_3$, CD_3OD or D_2O as solvents. The chemical shifts are expressed as ppm downfield from TMS. All melting points were determined with a Thomas-Hoover apparatus (A.H. Thomas Co.) and are uncorrected.

Purity of the compounds was checked using a Hewlett-Packard 1100 HPLC equipped with a Luna 5 μm RP-C18(2) analytical column (250 × 4.6 mm; Phenomenex, Torrance, CA). System A: linear gradient solvent system: H_2O–CH_3CN from 80: 20 to 0: 100 in 20 min, then isocratic for 5 min; the flow rate was 1 mL min^{-1}. System B: linear gradient solvent system: 5 mM TBAP–CH_3CN from 80: 20 to 20: 80 in 20 min, then isocratic for 2 min; the flow rate was 1 mL min^{-1}. Peaks were detected by UV absorption with a diode array detector. All derivatives tested for biological activity showed >98% purity in the HPLC systems.

TLC analysis was carried out on aluminium sheets precoated with silica gel F_{254} (0.2 mm) from Aldrich. Low-resolution CI–NH_3 (chemical ionization) mass spectra were measured with a Finnigan 4600 mass spectrometer, and low-resolution FAB (fast atom bombardment) mass spectrometry was performed with a JEOL SX102 spectrometer with 6 kV Xe atoms following desorption from a glycerol matrix or on LC/MS 1100 Agilent, 1100 MSD, with a Waters Atlantis C18 column. High-resolution mass measurements were performed on a Micromass/Waters LCT Premier Electrospray Time of Flight (TOF) mass spectometer coupled with a Waters HPLC system.

(2*S*)-3-Allyloxy-2-*tert*-butoxycarbonylamino-propionic acid methyl ester (3)— To a solution of compound **2** (4.48 g, 18.3 mmol) in 130 mL of Et_2O–MeOH (1: 1) was added $TMSCHN_2$ (2 M in Et_2O) dropwise until a yellow tint persisted. The reaction mixture was stirred at room temperature for 30 min. The solvent was removed *in vacuo*, and the residue was purified by silica gel column chromatography (petroleum ether–ethyl acetate, 15: 1) to afford **3** (3.71 g, 78%). ^{1}H NMR ($CDCl_3$) δ 5.83 (m, 1H, C*H*=CH_2), 5.38 (br d, J = 8.8 Hz, 1H, NH), 5.21 (m, 2H, CH=C*H*$_2$), 4.43 (m, 1H, H-2), 3.98 (m, 2H, C*H*$_2$CH=CH_2),

3.85 (m, 1H, 1H-3), 3.77 (s, 3H, OCH_3), 3.65 (m, 1H, 1H-3), 1.46 [s, 9H, $C(CH_3)_3$]; MS (FAB) *m*/*z* 260 $(M + H)^+$.

(2*S*)-3-Allyloxy-2-amino-propionic acid (4)—To a solution of compound **2** (242 mg, 0.99 mmol) in CH_2Cl_2 (15 mL) was added TFA (1 mL). The reaction mixture was stirred at room temperature for 2 h. The solvent was removed *in vacuo* to obtain compound **4** as a crude solid (520 mg), which then was used directly in the next step without further purification. ^{1}H NMR (D_2O) δ 5.96 (m, 1H, C*H*=CH_2), 5.33 (m, 2H, CH=C*H*$_2$), 4.11 (m, 2H, C*H*$_2$CH=CH_2), 3.93 (m, 3H, H-2, H-3).

(2*S*)-3-Allyloxy-2-amino-propionic acid methyl ester (5)—To a solution of compound **3** (1.02 g, 3.93 mmol) in CH_2Cl_2 (60 mL) was added TFA (10 mL). The reaction mixture was stirred at room temperature for 30 min, and then was neutralized with the addition of saturated $NaHCO_3$ (225 mL). The product was extracted with CH_2Cl_2, dried over Na_2SO_4, and evaporated to obtain compound **5** (585 mg, 93%). ^{1}H NMR ($CDCl_3$) δ 5.87 (m, 1H, C*H*=CH_2), 5.22 (m, 2H, CH=C*H*$_2$), 4.01 (m, 2H, C*H*$_2$CH=CH_2), 3.75 (s, 3H, OCH_3), 3.68 (m, 3H, H-2, H-3).

(2*R*)-3-Allylsulfanyl-2-amino-propionic acid methyl ester (8)—To a suspension of compound **7** (1.21 g, 7.49 mmol) in 55 mL of Et_2O–MeOH–CH_2Cl_2 (1:5:5), was added $TMSCHN_2$ (2 M in Et_2O) dropwise until a yellow tint persisted. The reaction mixture was stirred at room temperature for 2 h. The solvent was removed *in vacuo* to obtain compound **8** as a crude solid (1.46 g), which then was used directly in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ 5.78 (m, 1H, C*H*=CH_2), 5.12 (m, 2H, CH=C*H*$_2$), 3.75 (s, 3H, OCH_3), 3.64 (m, 1H, H-2), 3.15 (m, 2H, C*H*$_2$CH=CH_2), 2.88 (m, 1H, 1H-3), 2.71 (m, 1H, 1H-3); MS (CI) *m*/*z* 176 $(M + H)^+$.

(2*S*)-3-Allyloxy-2-(2,4-dimethoxybenzylamino)-propionic acid (9)—(**Procedure A**) To a solution containing the crude solid **4** (260 mg) in MeOH (20 mL) was added $NaBH_3CN$ (38 mg, 0.58 mmol) and 2,4-dimethoxybenzaldehyde (75 mg, 0.45 mmol). The reaction mixture was stirred at room temperature for 24 h. Water (3 mL) was added, and the product was extracted with CH_2Cl_2 and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography on silica gel (CH_2Cl_2–MeOH, 92: 8) to give **9** (77 mg, 53% from **2**) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.25 (d, *J* = 8.7 Hz, 1H, Ar–H), 6.47 (m, 2H, Ar–H), 5.83 (m, 1H, C*H*=CH_2), 5.19 (m, 2H, CH=C*H*$_2$), 4.76 (br s, 1H, NH), 4.39 (d, *J* = 13.0 Hz, 1H, *H*CHAr), 4.24 (d, *J* = 13.0 Hz, 1H, HC*H*Ar), 4.01–3.69 (m, 4H, C*H*$_2$CH=CH_2, H-3), 3.85 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.56 (m, 1H, H-2). HRMS *m*/*z* found 296.1501 $(M + H)^+$. $C_{15}H_{22}NO_5$ requires 296.1498. Mp 135–136 °C.

(2*S*)-3-Allyloxy-2-(2,4,6-trimethoxybenzylamino)-propionic acid (10)—(**Procedure A**) Compound **10** (84 mg, 53% from **2**) was obtained from a solution of crude solid **4** (260 mg), $NaBH_3CN$ (38 mg, 0.58 mmol) and 2,4,6-trimethoxybenz-aldehyde (89 mg, 0.45 mmol) in MeOH (20 mL). ^{1}H NMR ($CDCl_3$) δ 6.11 (s, 2H, Ar–H), 5.79 (m, 1H, C*H*=CH_2), 5.54 (br s, 1H, NH), 5.17 (m, 2H, CH=C*H*$_2$), 4.49 (d, *J* = 13.2 Hz, 1H, *H*CHAr), 4.40 (d, *J* = 13.2 Hz, 1H, HC*H*Ar), 3.98–3.74 (m, 3H, C*H*$_2$CH=CH_2, 1H-3), 3.82 (s, 9H, OCH_3), 3.68 (m, 1H, 1H-3), 3.54 (m, 1H, H-2). HRMS *m*/*z* found 326.1588 $(M + H)^+$. $C_{16}H_{24}NO_6$ requires 326.1604.

(2*R*)-3-Allylsulfanyl-2-(2,4-dimethoxybenzylamino)-propionic acid (11)—(**Procedure A**) Compound **11** (150 mg, 41%) as a white solid was obtained from **7** (206 mg, 1.28 mmol), $NaBH_3CN$ (100 mg, 1.51 mmol) and 2,4-dimethoxybenzaldehyde (197 mg, 1.16 mmol) in MeOH (40 mL). ^{1}H NMR ($CDCl_3$) δ 7.30 (d, *J* = 8.7 Hz, 1H, Ar–H), 6.81 (br

s, 1H, NH), 6.48 (m, 2H, Ar–H), 5.64 (m, 1H, C*H*=CH_2), 5.05 (m, 2H, CH=C*H*$_2$), 4.45 (d, *J* = 13.2 Hz, 1H, *H*CHAr), 4.18 (d, *J* = 13.2 Hz, 1H, HC*H*Ar), 3.87 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.41 (m, 1H, H-2), 3.16 (m, 1H, *H*CHCH=CH_2), 2.92 (m, 3H, HC*H*CH=CH_2, H-3). HRMS *m*/*z* found 312.1265 $(M + H)^+$. $C_{15}H_{22}NO_4S$ requires 312.1270. Mp 150–151 °C.

(2*R*)-3-Allylsulfanyl-2-(2,4,6-trimethoxybenzylamino)-propionic acid (12)— (**Procedure A**) Compound **12** (168 mg, 41%) was obtained from **7** (214 mg, 1.33 mmol), $NaBH_3CN$ (104 mg, 1.57 mmol) and 2,4,6-trimethoxybenzaldehyde (242 mg, 1.21 mmol) in MeOH (40 mL). ^{1}H NMR ($CDCl_3$) δ 6.13 (s, 2H, Ar–H), 6.06 (br s, 1H, NH), 5.61 (m, 1H, C*H*=CH_2), 5.06 (m, 2H, CH=C*H*$_2$), 4.49 (d, *J* = 13.3 Hz, 1H, *H*CHAr), 4.41 (d, *J* = 13.3 Hz, 1H, HC*H*Ar), 3.85 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3), 3.34 (m, 1H, H-2), 3.17 (m, 1H, *H*CHCH=CH_2), 2.84 (m, 3H, HC*H*CH=CH_2, H-3). MS (API-ES) *m*/*z* 342 $(M + H)^+$.

(2*S*)-3-Allyloxy-2-(2,4-dimethoxybenzylamino)-propionic acid methyl ester (13)—(**Procedure B**) To a solution of compound **5** (169 mg, 1.06 mmol) in CH_2Cl_2 (30 mL) was added $NaBH(OAc)_3$ (280 mg, 1.26 mmol) and 2,4-dimethoxybenzaldehyde (164 mg, 0.97 mmol). The reaction mixture was stirred at room temperature for 2 h. Water (30 mL) was added, the product was extracted with CH_2Cl_2 and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography on silica gel (petroleum ether–ethyl acetate, 2: 1) to give **13** (253 mg, 85%). (**Procedure C**) To a suspension of compound **9** (23 mg, 0.08 mmol) in 5.5 mL of Et_2O–MeOH–CH_2Cl_2 (1: 5: 5) was added $TMSCHN_2$ (2 M in Et_2O) until a yellow tint persisted. The reaction mixture was stirred at room temperature for 30 min. After removal of the solvent, the product was purified by preparative thin-layer chromatography (petroleum ether–ethyl acetate, 2: 1) to give **13** (8 mg, 33%). ^{1}H NMR ($CDCl_3$) δ 7.14 (d, *J* = 8.7 Hz, 1H, Ar–H), 6.42 (m, 2H, Ar–H), 5.84 (m, 1H, C*H*=CH_2), 5.19 (m, 2H, CH=C*H*$_2$), 3.95 (m, 2H, C*H*$_2$CH=CH_2), 3.80 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.75 (d, *J* = 8.4 Hz, 2H, C*H*$_2$Ar), 3.69 (s, 3H, OCH_3), 3.63 (m, 2H, H-3), 3.49 (m, 1H, H-2), 2.12 (br s, 1H, NH); MS (FAB) *m*/*z* 310 $(M + H)^+$.

(2*S*)-3-Allyloxy-2-(2,4,6-trimethoxybenzylamino)-propionic acid methyl ester (14)—(**Procedure B**) Compound **14** (934 mg, 82%) was obtained from **5** (585 mg, 3.68 mmol), $NaBH(OAc)_3$ (970 mg, 4.35 mmol) and 2,4,6-trimethoxybenzaldehyde (670 mg, 3.34 mmol) in CH_2Cl_2 (70 mL). (**Procedure C**) Compound **14** (12 mg, 32%) was obtained from **10** (36 mg, 0.11 mmol) and $TMSCHN_2$ (2 M in Et_2O) in Et_2O–MeOH–CH_2Cl_2 (1: 5: 5) (5.5 mL). ^{1}H NMR ($CDCl_3$) δ 6.09 (s, 2H, Ar–H), 5.83 (m, 1H, C*H*=CH_2), 5.17 (m, 2H, CH=C*H*$_2$), 3.92 (m, 2H, C*H*$_2$CH=CH_2), 3.83 (d, *J* = 2.1 Hz, 2H, C*H*$_2$Ar), 3.80 (s, 3H, OCH_3), 3.79 (s, 6H, OCH_3), 3.63 (s, 3H, OCH_3), 3.59 (m, 2H, H-3), 3.50 (m, 1H, H-2), 2.71 (br s, 1H, NH); MS (FAB) *m*/*z* 340 $(M + H)^+$.

(2*R*)-3-Allylsulfanyl-2-(2,4-dimethoxybenzylamino)-propionic acid methyl ester (15)—(**Procedure B**) Compound **15** (797 mg, 74% from **7**) was obtained from a solution containing crude solid **8** (731 mg), $NaBH(OAc)_3$ (988 mg, 4.43 mmol) and 2,4-dimethoxybenzaldehyde (578 mg, 3.41 mmol) in CH_2Cl_2 (65 mL). The product was purified by column chromatography on silica gel (petroleum ether–ethyl acetate, 3: 1). (**Procedure C**) Compound **15** (76 mg, 55%) was obtained from **11** (132 mg, 0.42 mmol) and $TMSCHN_2$ (2 M in Et_2O) in Et_2O–MeOH (1: 1) (10 mL). The product was purified by column chromatography on silica gel (petroleum ether–ethyl acetate, 4: 1). ^{1}H NMR ($CDCl_3$) δ 7.12 (d, *J* = 8.7 Hz, 1H, Ar–H), 6.42 (m, 2H, Ar–H), 5.73 (m, 1H, C*H*=CH_2), 5.06 (m, 2H, CH=C*H*$_2$), 3.81 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.76 (m, 2H, C*H*$_2$Ar), 3.69 (s, 3H, OCH_3), 3.41 (m, 1H, H-2), 3.05 (m, 2H, C*H*$_2$CH=CH_2), 2.74 (m, 2H, H-3); MS (CI) *m*/*z* 326 $(M + H)^+$.

(2*R*)-3-Allylsulfanyl-2-(2,4,6-trimethoxybenzylamino)-propionic acid methyl ester (16)—(**Procedure B**) Compound **16** (697 mg, 58% from **7**) was obtained from a solution containing crude solid **8** (731 mg), $NaBH(OAc)_3$ (988 mg, 4.43 mmol) and 2,4,6-trimethoxybenzaldehyde (682 mg, 3.41 mmol) in CH_2Cl_2 (65 mL). The product was purified by column chromatography on silica gel (petroleum ether–ethyl acetate, 2: 1). (**Procedure C**) Compound **16** (35 mg, 22%) was obtained from **12** (154 mg, 0.45 mmol) and $TMSCHN_2$ (2M in Et_2O) in Et_2O–MeOH (1: 1) (12 mL). The product was purified by column chromatography on silica gel (petroleum ether–ethyl acetate, 4: 1). ^{1}H NMR ($CDCl_3$) δ 6.10 (s, 2H, Ar–H), 5.72 (m, 1H, C*H*=CH_2), 5.05 (m, 2H, CH=C*H*$_2$), 3.81 (m, 2H, C*H*$_2$Ar), 3.80 (s, 3H, OCH_3), 3.79 (s, 6H, OCH_3), 3.65 (s, 3H, OCH_3), 3.37 (m, 1H, H-2), 3.03 (m, 2H, C*H*$_2$CH=CH_2), 2.72 (m, 2H, H-3), 2.42 (br s, 1H, NH); MS (CI) *m*/*z* 356 $(M + H)^+$.

(2*S*)-(9*H* -Fluoren-9-ylmethoxycarbonylamino)-pent-4-enoic acid (17)—A solution of Fmoc-Cl (900 mg, 3.38 mmol) in dioxane (5 mL) was added, at 0 °C, to a suspension of L-allylglycine (299 mg, 2.60 mmol) and $NaHCO_3$ (655 mg, 7.79 mmol) in 16.8 mL H_2O–dioxane (1.4: 1). The mixture was stirred for 15 min at 0 °C and then at room temperature for 4.5 h. The pH was adjusted to approximately 9 with the addition of solid $NaHCO_3$ and the mixture was diluted with H_2O (120 mL) and washed with Et_2O. The aqueous layer was acidified to pH = 3 with an aqueous HCl solution (6 M). The product was extracted with EtOAc, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography on silica gel (CH_2Cl_2–MeOH, 97: 3) to afford **17** (615 mg, 70%). ^{1}H NMR ($CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H, Ar–H), 7.57 (d, *J* = 6.9 Hz, 2H, Ar–H), 7.39 (t, *J* = 7.4 Hz, 2H, Ar–H), 7.29 (t, *J* = 7.4 Hz, 2H, Ar–H), 5.71 (m, 1H, H-4), 5.34 (br d, *J* = 7.8 Hz, 1H, NH), 5.17 (m, 2H, H-5), 4.47 (m, 1H, H-2), 4.39 (d, *J* = 6.9 Hz, 2H, CHC*H*$_2$), 4.21 (t, *J* = 6.9 Hz, 1H, C*H*CH_2), 2.58 (m, 2H, H-3); MS (FAB) *m*/*z* 338 $(M + H)^+$.

(2*S*,2′*S*)-3-Allyloxy-2-*{*(2,4-dimethoxybenzyl)-[2′-(9*H*-fluoren-9-ylmethoxycarbonylamino)-pent-4′-enoyl]-amino*}*-propionic acid methyl ester (18)—(**Procedure D**) A solution containing compound **17** (638 mg, 1.89 mmol), HATU (742 mg, 1.89 mmol), HOAt (3.79 mL, 1.89 mmol, solution 0.5–0.7 M in DMF) and NEM (0.49 mL, 3.79 mmol) in CH_2Cl_2 (34.2 mL) was added to a solution of compound **13** (117 mg, 0.38 mmol) in 15.5 mL CH_2Cl_2–DMF (9: 1). The reaction mixture was stirred at room temperature overnight. The solvent was removed *in vacuo*, and the residue was purified by silica gel column chromatography (petroleum ether–ethyl acetate, 2: 1) to afford **18** (126 mg, 53%). ^{1}H NMR ($CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H, Ar–H), 7.61 (d, *J* = 7.2 Hz, 2H, Ar–H), 7.40 (t, *J* = 7.4 Hz, 2H, Ar–H), 7.31 (t, *J* = 7.4 Hz, 2H, Ar–H), 7.17 (d, *J* = 7.8 Hz, 1H, Ar–H), 6.43 (m, 2H, Ar–H), 5.74 (m, 2H, C*H*=CH_2), 5.14 (m, 4H, CH=C*H*$_2$), 4.80 (m, 1H, *H*CHAr), 4.53–4.02 (m, 6H, HC*H*Ar, CHC*H*$_2$OCO, C*H*CH_2OCO, C*H*CH_2CH=CH_2, H-2), 3.88 (m, 2H, OC*H*$_2$CH=CH_2), 3.84–3.55 (m, 2H, H-3), 3.79 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.59 (s, 3H, OCH_3), 2.48 (m, 2H, C*H*$_2$CH=CH_2); MS (FAB) *m*/*z* 629 $(M + H)^+$.

(3*S*,6*S*)-4-(2,4-Dimethoxybenzyl)-6-(9*H* -fluoren-9-ylmethoxy-carbonylamino)-5-oxo-3,4,5,6,7,10-hexahydro-2*H*-[1,4]oxazecine-3-carboxylic acid methyl ester (19)—(**Procedure E**) To a solution of compound **18** (70 mg, 0.11 mmol) in CH_2Cl_2 (500 mL) was added second generation Grubbs catalyst (35 mg, 0.04 mmol). The reaction mixture was stirred at room temperature for 48 h. The solvent was removed *in vacuo*, and the residue was purified by column chromatography on silica gel (petroleum ether–ethyl acetate, 2: 1) to afford **19** (40 mg, 60%). ^{1}H NMR ($CDCl_3$) δ 7.78 (d, *J* = 7.2 Hz, 2H, Ar–H), 7.63 (d, *J* = 7.2 Hz, 2H, Ar–H), 7.41 (t, *J* = 7.2 Hz, 2H, Ar–H), 7.32

(t, J = 7.1 Hz, 2H, Ar–H), 7.08 (d, J = 8.1 Hz, 1H, Ar–H), 6.40 (m, 2H, Ar–H), 6.07 (d, J = 7.5 Hz, 1H, NH), 5.83 (m, 2H, H-8, H-9), 5.25 (m, 1H, H-6), 4.94 (d, J = 15.0 Hz, 1H, *H*CHAr), 4.51–4.34 (m, 3H, CHC*H*$_2$OCO, 1H-10), 4.29–4.08 (m, 3H, C*H*CH$_2$OCO, H-2), 3.90 (m, 2H, HC*H*Ar, 1H-10), 3.79 (s, 3H, OCH$_3$), 3.75 (m, 1H, H-3), 3.69 (s, 3H, OCH$_3$), 3.43 (s, 3H, OCH$_3$), 2.68 (m, 1H, 1H-7), 2.53 (m, 1H, 1H-7); MS (FAB) m/z 601 (M + H)$^+$.

(1*S*,8*S*)-11-(2,4-Dimethoxybenzyl)-3-oxa-9,11-diazabicyclo-[6.2.2]dodec-5-ene-10,12-dione (20)—(**Procedure F**) Compound **19** (24 mg, 0.04 mmol) was stirred at room temperature in 5 mL of CH$_2$Cl$_2$–piperidine (4: 1) for 2 h. After the solvent was removed, the resulting residue was purified by silica gel column chromatography (CH$_2$Cl$_2$–MeOH, 96: 4) to obtain **20** (13 mg, 93%) as a white solid. ^{1}H NMR (CDCl$_3$) δ 7.24 (m, 1H, Ar–H), 6.45 (m, 2H, Ar–H), 5.93 (br s, 1H, NH), 5.62 (m, 2H, H-5, H-6), 4.98 (d, J = 14.3 Hz, 1H, *H*CHAr), 4.25 (m, 3H, H-4, H-8), 4.14 (d, J = 14.3 Hz, 1H, HC*H*Ar), 3.89 (m, 3H, H-1, H-2), 3.79 (s, 6H, OCH$_3$), 3.11 (m, 1H, 1H-7), 2.83 (m, 1H, 1H-7); ^{13}C NMR (CDCl$_3$) δ 169.5, 168.1, 161.0, 158.9, 132.0, 131.4, 125.9, 116.4, 104.9, 98.6, 73.2, 71.5, 61.5, 55.6, 53.8, 41.6, 34.0; HRMS m/z found 347.1591 (M + H)$^+$. $C_{18}H_{23}N_2O_5$ requires 347.1607; HPLC (System A) 8.1 min (99%), (System B) 9.1 min (99%). Mp 155–156 °C.

(1*S*,8*S*)-11-(2,4-Dimethoxybenzyl)-9-methyl-3-oxa-9,11-diaza-bicyclo[6.2.2]dodec-5-ene-10,12-dione (21)—(**Procedure G**) A solution of compound **20** (10 mg, 0.03 mmol) in THF (1 mL) was added, at 0 °C, to a suspension of NaH (3.5 mg, 0.09 mmol, 60% dispersion in mineral oil) in THF (1 mL). After stirring at room temperature for 30 min, the reaction mixture was cooled to 0 °C and CH$_3$I (18 μL, 0.29 mmol) was added. The reaction mixture was stirred at room temperature for 5 h, followed by quenching with H$_2$O. The product was extracted with CH$_2$Cl$_2$, dried over Na$_2$SO$_4$, and the solvent was removed under reduced pressure. The resulting residue was purified by preparative thin-layer chromatography (CH$_2$Cl$_2$–MeOH, 96: 4) to obtain **21** (6.6 mg, 63%). ^{1}H NMR (CDCl$_3$) δ 7.25 (m, 1H, Ar–H), 6.44 (m, 2H, Ar–H), 5.67 (m, 1H, H-6), 5.55 (m, 1H, H-5), 4.95 (d, J = 14.4 Hz, 1H, *H*CHAr), 4.21 (m, 2H, H-4), 4.16 (d, J = 14.4 Hz, 1H, HC*H*Ar), 4.15 (m, 1H, H-8), 3.90 (m, 3H, H-1, H-2), 3.79 (s, 6H, OCH$_3$), 3.07 (m, 1H, 1H-7), 2.91 (s, 3H, NCH$_3$), 2.88 (m, 1H, 1H-7); ^{13}C NMR (CDCl$_3$) δ 168.1, 167.3, 161.0, 158.9, 132.1, 130.8, 125.7, 116.5, 104.9, 98.6, 72.8, 71.4, 61.7, 61.1, 55.6, 41.3, 33.9, 32.9; HRMS m/z found 377.1777 (M+ H)$^+$. $C_{19}H_{25}N_2O_5$ requires 361.1763; HPLC (System A) 9.1 min (98%), (System B) 10.4 min (98%).

(1*S*,8*S*)-9-Benzyl-11-(2,4-dimethoxybenzyl)-3-oxa-9,11-diaza-bicyclo[6.2.2]dodec-5-ene-10,12-dione (22)—(**Procedure G**) Compound **22** (7.5 mg, 60%) was obtained as a white solid from **20** (10 mg, 0.03 mmol), NaH (4.2 mg, 0.10 mmol, 60% dispersion in mineral oil) and BnBr (54 μL, 0.45 mmol) in THF (2 mL) after for 8 h stirring at room temperature. The product was purified by preparative thin-layer chromatography (CH$_2$Cl$_2$–MeOH, 97: 3). ^{1}H NMR (CDCl$_3$) δ 7.29 (m, 3H, Ar–H), 7.19 (m, 3H, Ar–H), 6.44 (m, 2H, Ar–H), 5.68 (m, 1H, H-6), 5.55 (m, 1H, H-5), 5.29 (d, J = 14.7 Hz, 1H, *H*CHPh), 4.96 (d, J = 14.7 Hz, 1H, *H*CHAr), 4.20 (m, 2H, H-4), 4.16 (m, 2H, H-8, HC*H*Ar), 3.99 (m, 2H, H-1, 1H-2), 3.80 (s, 3H, OCH$_3$), 3.79 (m, 2H, 1H-2, HC*H*Ph), 3.77 (s, 3H, OCH$_3$), 2.89 (m, 2H, H-7); ^{13}C NMR (CDCl$_3$) δ 167.9, 167.4, 161.1, 158.9, 135.7, 131.8, 130.5, 129.1, 128.4, 128.1, 126.3, 116.4, 104.9, 98.7, 72.8, 71.5, 61.7, 57.8, 55.6 (2C), 48.7, 41.7, 32.9; HRMS m/z found 437.2095 (M + H)$^+$. $C_{25}H_{29}N_2O_5$ requires 437.2076; HPLC (System A) 13.4 min (98%), (System B) 16.2 min (98%). Mp 129–130 °C.

(2*S*,2′*S*)-3-Allyloxy-2-[[2′-(9*H* -fluoren-9-ylmethoxycarbonyl-amino)-pent-4′-enoyl]-(2,4,6-trimethoxybenzyl)-amino]-propionic acid methyl ester (23)—(**Procedure D**) Compound **23** (278 mg, 40%) was obtained from **14** (357 mg, 1.05 mmol) in

45 mL CH_2Cl_2–DMF (9: 1), and **17** (1.77 g, 5.26 mmol), HATU (2.06 g, 5.26 mmol), HOAt (10.5 mL, 5.26 mmol, solution 0.5–0.7 M in DMF) and NEM (1.35 mL, 10.5 mmol), in CH_2Cl_2 (99 mL) and DMF (0.47 mL). ^{1}H NMR ($CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2H, Ar–H), 7.61 (d, J = 7.5 Hz, 2H, Ar–H), 7.40 (t, J = 7.2 Hz, 2H, Ar–H), 7.30 (t, J = 7.2 Hz, 2H, Ar–H), 6.10 (s, 2H, Ar–H), 5.84 (m, 2H, C*H*=CH_2), 5.15 (m, 4H, CH=C*H*$_2$), 4.61 (m, 2H, C*H*$_2$Ar), 4.49–4.07 (m, 5H, CHC*H*$_2$OCO, C*H*CH_2OCO, C*H*CH_2CH=CH_2, H-2), 3.98 (m, 2H, OC*H*$_2$CH=CH_2), 3.81 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.70 (m, 2H, H-3), 3.50 (s, 3H, OCH_3), 2.58 (m, 1H, *H*CHCH=CH_2), 2.44 (m, 1H, HC*H* CH=CH_2); MS (API-ES) m/z 681 $(M + Na)^+$.

(3*S*,6*S*)-6-(9*H* -Fluoren-9-ylmethoxycarbonylamino)-5-oxo-4-(2,4,6-trimethoxybenzyl)-3,4,5,6,7,10-hexahydro-2*H* -[1,4]oxa-zecine-3-carboxylic acid methyl ester (24)—(**Procedure E**) Compound **24** (5.5 mg, 57%) was obtained from **23** (10 mg, 0.015 mmol) and second generation Grubbs catalyst (4 mg, 0.005 mmol) in CH_2Cl_2 (60 mL). The product was purified by preparative thin-layer chromatography (petroleum ether–ethyl acetate, 2: 1). ^{1}H NMR ($CDCl_3$) δ 7.78 (d, J = 7.5 Hz, 2H, Ar–H), 7.63 (d, J = 7.5 Hz, 2H, Ar–H), 7.41 (t, J = 7.4 Hz, 2H, Ar–H), 7.32 (t, J = 7.4 Hz, 2H, Ar–H), 6.08 (m, 3H, Ar–H, NH), 5.83 (m, 2H, H-8, H-9), 5.33 (m, 1H, H-6), 4.78–4.37 (m, 5H, C*H*$_2$Ar, CHC*H*$_2$OCO, 1H-10), 4.29–4.08 (m, 3H, C*H*CH_2OCO, H-2), 3.92 (m, 2H, H-3, 1H-10), 3.80 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 3.40 (s, 3H, OCH_3), 2.70 (m, 1H, 1H-7), 2.49 (m, 1H, 1H-7); MS (API-ES) m/z 653 $(M + Na)^+$.

(1*S*,8*S*)-11-(2,4,6-Trimethoxybenzyl)-3-oxa-9,11-diazabicyclo-[6.2.2]dodec-5-ene-10,12-dione (25)—(**Procedure F**) Compound **25** (35 mg, 73%) was obtained as a white solid from **24** (80 mg, 0.13 mmol) and 15 mL of CH_2Cl_2–piperidine (4: 1). ^{1}H NMR ($CDCl_3$) δ 6.17 (br s, 1H, NH), 6.09 (s, 2H, Ar–H), 5.63 (m, 2H, H-5, H-6), 5.26 (d, J = 14.1 Hz, 1H, *H*CHAr), 4.29 (m, 2H, H-4), 4.24 (m, 1H, H-8), 4.10 (d, J = 14.1 Hz, 1H, *H*CHAr), 4.04 (m, 1H, 1H-2), 3.81 (s, 3H, OCH_3), 3.77 (s, 6H, OCH_3), 3.74 (m, 1H, 1H-2), 3.64 (m, 1H, H-1), 3.11 (m, 1H, 1H-7), 2.81 (m, 1H, 1H-7); ^{13}C NMR ($CDCl_3$) δ 169.9, 167.7, 161.8, 160.3, 131.5, 126.0, 103.2, 90.7, 73.0, 71.4, 59.7, 55.9, 55.6, 53.8, 35.5, 34.1; HRMS m/z found 377.1705 $(M + H)^+$. $C_{19}H_{25}N_2O_6$ requires 377.1713; HPLC (System A) 8.3 min (99%), (System B) 9.3 min (99%). Mp 123–124 °C.

(1*S*,8*S*)-3-Oxa-9,11-diazabicyclo[6.2.2]dodec-5-ene-10,12-dione (26)—(**Procedure H**) To a solution of compound **25** (10 mg, 0.03 mmol) in CH_2Cl_2 (1.5 mL) was added TFA (0.75 mL). After stirring at room temperature for 7 h, the solvent was removed under reduced pressure. The residue was purified by preparative thin-layer chromatography (CH_2Cl_2–MeOH, 94: 6) to afford **26** (5 mg, 96%) as a white solid. ^{1}H NMR (CD_3OD) δ 5.59 (m, 2H, H-5, H-6), 4.33 (m, 2H, H-4), 4.06 (m, 1H, H-8), 3.95 (m, 1H, 1H-2), 3.77 (m, 2H, H-1, 1H-2), 3.04 (m, 1H, 1H-7), 2.77 (m, 1H, 1H-7); ^{13}C NMR (CD_3OD) δ 173.0, 171.1, 132.5, 126.2, 76.0, 74.9, 59.1, 54.4, 35.0; HRMS m/z found 197.0932 $(M + H)^+$. $C_9H_{13}N_2O_3$ requires 197.0926.

(1*S*,8*S*)-9-Methyl-11-(2,4,6-trimethoxybenzyl)-3-oxa-9,11-diazabicyclo[6.2.2]dodec-5-ene-10,12-dione (27)—(**Procedure G**) Compound **27** (10 mg, 96%) was obtained from **25** (10 mg, 0.03 mmol), NaH (3.5 mg, 0.09 mmol, 60% dispersion in mineral oil) and CH_3I (18 μL, 0.29 mmol) in THF (2 mL) after 7 h of stirring at room temperature. The product was purified by preparative thin-layer chromatography (CH_2Cl_2–MeOH, 96: 4). ^{1}H NMR ($CDCl_3$) δ 6.08 (s, 2H, Ar–H), 5.68 (m, 1H, H-6), 5.57 (m, 1H, H-5), 5.23 (d, J = 13.8 Hz, 1H, *H*CHAr), 4.24 (m, 2H, H-4), 4.15 (m, 1H, H-8), 4.10 (d, J = 13.8 Hz, 1H, HC*H*Ar), 3.96 (m, 1H, 1H-2), 3.80 (s, 3H, OCH_3), 3.78 (m, 1H, 1H-2), 3.77 (s, 6H, OCH_3), 3.69 (m, 1H, H-1), 3.08 (m, 1H, 1H-7), 2.89 (s, 3H, NCH_3), 2.86 (m,

1H, 1H-7); ^{13}C NMR ($CDCl_3$) δ 167.7, 167.6, 161.8, 160.3, 130.7, 126.0, 103.2, 90.7, 72.5, 71.2, 61.1, 60.0, 55.9, 55.6, 35.2, 33.8, 32.9; HRMS *m*/*z* found 391.1867 $(M + H)^+$. $C_{20}H_{27}N_2O_6$ requires 391.1869; HPLC (System A) 9.1 min (98%), (System B) 10.6 min (98%). Mp 158–159 °C.

(1*S*,8*S*)-9-Benzyl-11-(2,4,6-trimethoxybenzyl)-3-oxa-9,11-diazabicyclo[6.2.2]dodec-5-ene-10,12-dione (28)—(**Procedure G**) Compound **28** (10.6 mg, 85%) was obtained from **25** (10 mg, 0.03 mmol), NaH (4 mg, 0.1 mmol, 60% dispersion in mineral oil) and BnBr (64 μL, 0.53 mmol) in THF (2 mL) after 24 h of stirring at room temperature. The product was purified by preparative thin-layer chromatography (CH_2Cl_2–MeOH, 97: 3). ^{1}H NMR ($CDCl_3$) δ 7.28 (m, 3H, Ar–H), 7.16 (m, 2H, Ar–H), 6.10 (s, 2H, Ar–H), 5.70 (m, 1H, H-6), 5.60 (m, 1H, H-5), 5.33 (d, *J* = 15.3 Hz, 1H, *H*CHPh), 5.20 (d, *J* = 14.0 Hz, 1H, *H*CHAr), 4.27 (m, 2H, H-4), 4.15 (m, 1H, H-8), 4.13 (d, *J* =14.0 Hz, 1H, HC*H*Ar), 3.89 (m, 2H, H-2), 3.82 (s, 3H, OCH_3), 3.75 (s, 6H, OCH_3), 3.73 (m, 1H, H-1), 3.70 (d, *J* = 15.3 Hz, 1H, HC*H*Ph), 2.98 (m, 1H, 1H-7), 2.86 (m, 1H, 1H-7); ^{13}C NMR ($CDCl_3$) δ 167.9, 167.7, 161.8, 160.3, 135.8, 130.6, 128.9, 128.1, 128.0, 126.5, 103.1, 90.7, 72.4, 71.1, 59.9, 57.7, 55.9, 55.6, 48.4, 35.3, 32.8; HRMS *m*/*z* found 467.2208 $(M + H)^+$. $C_{26}H_{31}N_2O_6$ requires 467.2182; HPLC (System A) 13.3 min (98%), (System B) 16.3 min (98%).

(1*S*,8*S*)-9-Methyl-3-oxa-9,11-diazabicyclo[6.2.2]dodec-5-ene-10,12-dione (29)—(**Procedure H**) Compound **29** (1.7 mg, 63%) was obtained from **27** (5 mg, 0.01 mmol) and TFA (0.35 mL) in CH_2Cl_2 (0.7 mL). ^{1}H NMR ($CDCl_3$) δ 6.12 (br s, 1H, NH), 5.62 (m, 2H, H-5, H-6), 4.32 (m, 2H, H-4), 4.07 (m, 2H, 1H-2, H-8), 3.96 (m, 1H, H-1), 3.73 (m, 1H, 1H-2), 3.14 (m, 1H, 1H-7), 2.95 (s, 3H, NCH_3), 2.88 (m, 1H, 1H-7); HRMS *m*/*z* found 211.1086 $(M+ H)^+$. $C_{10}H_{15}N_2O_3$ requires 211.1083.

(1*S*,8*S*)-9-Benzyl-3-oxa-9,11-diazabicyclo[6.2.2]dodec-5-ene-10,12-dione (30)—(**Procedure H**) Compound **30** (2.7 mg, 89%) was obtained from **28** (5 mg, 0.01 mmol) and TFA (0.35 mL) in CH_2Cl_2 (0.7 mL). The product was purified by preparative thin-layer chromatography (CH_2Cl_2–MeOH, 96: 4). ^{1}H NMR ($CDCl_3$) δ 7.31 (m, 3H, Ar–H), 7.25 (m, 2H, Ar–H), 6.22 (br s, 1H, NH), 5.64 (m, 2H, H-5, H-6), 5.38 (d, *J* = 15.0 Hz, 1H, *H*CHPh), 4.35 (m, 2H, H-4), 4.11 (m, 2H, 1H-2, H-8), 4.04 (m, 1H, H-1), 3.73 (d, *J* = 15.0 Hz, 1H, HC*H*Ph), 3.70 (m, 1H, 1H-2), 3.01 (m, 1H, 1H-7), 2.86 (m, 1H, 1H-7); HRMS *m*/*z* found 287.1391 $(M + H)^+$. $C_{16}H_{19}N_2O_3$ requires 287.1396.

(2*R*,2′*S*)-3-Allylsulfanyl-2-*{*(2,4-dimethoxybenzyl)-[2′-(9*H* -fluoren-9 -ylmethoxycarbonylamino) - pent - 4′ - enoyl] - amino *}* -propionic acid methyl ester (31)—(**Procedure D**) Compound **31** (107 mg, 37%) was obtained from **15** (146 mg, 0.45 mmol) in 20 mL CH_2Cl_2–DMF (9: 1), and **17** (755 mg, 2.24 mmol), HATU (878 mg, 2.24 mmol), HOAt (4.48 mL, 2.24 mmol, solution 0.5–0.7 M in DMF) and NEM (0.58 mL, 4.48 mmol) in CH_2Cl_2 (40.3 mL). The product was purified by column chromatography on silica gel (petroleum ether–ethyl acetate, 3: 1). ^{1}H NMR ($CDCl_3$) δ 7.77 (d, *J* = 7.2 Hz, 2H, Ar–H), 7.61 (d, *J* = 7.2 Hz, 2H, Ar–H), 7.40 (t, *J* = 7.4 Hz, 2H, Ar–H), 7.31 (t, *J* = 7.4 Hz, 2H, Ar–H), 7.16 (d, *J* = 8.7 Hz, 1H, Ar–H), 6.43 (m, 2H, Ar–H), 5.75 (m, 2H, C*H*=CH_2), 5.11 (m, 4H, CH=C*H*$_2$), 4.78 (d, *J* = 15.6 Hz, 1H, *H*CHAr), 4.49–4.18 (m, 5H, HC*H*Ar, CHC*H*$_2$OCO, C*H*CH_2OCO, C*H*CH_2CH=CH_2), 4.07 (m, 1H, H-2), 3.78 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.55 (s, 3H, OCH_3), 3.10 (m, 3H, SC*H*$_2$CH=CH_2, 1H-3), 2.94 (m, 1H, 1H-3), 2.61 (m, 1H, *H*CHCH=CH_2), 2.46 (m, 1H, HC*H*CH=CH_2); MS (API-ES) *m*/*z* 667 $(M + Na)^+$.

(3*R*,6*S*)-4-(2,4-Dimethoxybenzyl)-6-(9*H* -fluoren-9-ylmethoxy-carbonylamino)-5-oxo-3,4,5,6,7,10-hexahydro-2*H*-[1,4]thiazecine-3-carboxylic acid methyl ester (32)—(**Procedure E**) Compound **32** (52 mg, 57%) was obtained from **31** (95 mg, 0.15 mmol) and second generation Grubbs catalyst (46 mg, 0.05 mmol) in CH_2Cl_2 (148 mL). The product was purified by column chromatography on silica gel (petroleum ether–ethyl acetate, 2.5: 1). ^{1}H NMR ($CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H, Ar–H), 7.63 (d, J = 7.5 Hz, 2H, Ar–H), 7.41 (t, J = 7.4 Hz, 2H, Ar–H), 7.32 (t, J = 7.5 Hz, 2H, Ar–H), 7.12 (d, J = 7.5 Hz, 1H, Ar–H), 6.41 (m, 2H, Ar–H), 6.15 (br d, J = 6.3 Hz, 1H, NH), 5.67 (m, 2H, H-8, H-9), 5.30 (m, 1H, H-6), 5.05 (d, J = 14.4 Hz, 1H, *H*CHAr), 4.43 (m, 2H, CHC*H*$_2$OCO), 4.29–4.07 (m, 2H, C*H*CH$_2$OCO, HC*H* Ar), 3.79 (s, 3H, OCH_3), 3.76–3.63 (m, 1H, H-3), 3.71 (s, 3H, OCH_3), 3.52–3.28 (m, 2H, H-10), 3.37 (s, 3H, OCH_3), 3.02–2.72 (m, 3H, H-2, 1H-7), 2.45 (m, 1H, 1H-7); MS (API-ES) m/z 639 $(M + Na)^+$.

(1*R*,8*S*)-11-(2,4-Dimethoxybenzyl)-3-thia-9,11-diazabicyclo-[6.2.2]dodec-5-ene-10,12-dione (33)—(**Procedure F**) Compound **33** (19 mg, 73%) was obtained from **32** (45 mg, 0.07 mmol) and 5 mL of CH_2Cl_2–piperidine (4: 1). The product was purified by preparative thin-layer chromatography (CH_2Cl_2–MeOH, 96: 4). ^{1}H NMR ($CDCl_3$) δ 7.26 (d, J = 8.4 Hz, 1H, Ar–H), 6.45 (m, 3H, Ar–H, NH), 5.71 (m, 2H, H-5, H-6), 5.21 (d, J = 14.6 Hz, 1H, *H*CHAr), 4.33 (m, 1H, H-1), 4.28 (d, J = 14.6 Hz, 1H, HC*H*Ar), 4.24 (m, 1H, H-2), 3.79 (s, 6H, OCH_3), 3.45 (m, 1H, 1H-4), 3.25–3.02 (m, 4H, H-2, 1H-4, 1H-7), 2.55 (m, 1H, 1H-7); ^{13}C NMR ($CDCl_3$) δ 169.6, 167.0, 161.1, 159.1, 132.5, 130.7, 127.4, 116.2, 104.7, 98.7, 60.1, 56.9, 55.6, 55.2, 45.4, 32.4, 30.4, 29.3; HRMS m/z found 363.1371 $(M + H)^+$. $C_{18}H_{23}N_2O_4S$ requires 363.1379; HPLC (System A) 9.8 min (99%), (System B) 11.4 min (99%).

(2*R*,2′*S*)-3-Allylsulfanyl-2-[[2′ -(9*H* -fluoren-9-ylmethoxycar-bonylamino)-pent-4′-enoyl]-(2,4,6-trimethoxybenzyl)-amino]-propionic acid methyl ester (34)—(**Procedure D**) Compound **34** (124 mg, 41%) was obtained from **16** (159 mg, 0.45 mmol) in 20 mL CH_2Cl_2–DMF (9: 1), and **17** (755 mg, 2.24 mmol), HATU (878 mg, 2.24 mmol), HOAt (4.48 mL, 2.24 mmol, solution 0.5–0.7 M in DMF) and NEM (0.58 mL, 4.48 mmol) in CH_2Cl_2 (40.3 mL). The product was purified by column chromatography on silica gel (petroleum ether–ethyl acetate, 2.5: 1). ^{1}H NMR ($CDCl_3$) δ 7.76 (d, J = 7.5 Hz, 2H, Ar–H), 7.62 (m, 2H, Ar–H), 7.40 (t, J = 7.2 Hz, 2H, Ar–H), 7.30 (m, 2H, Ar–H), 6.10 (s, 2H, Ar–H), 5.82 (m, 2H, C*H*=CH$_2$), 5.13 (m, 4H, CH=C*H*$_2$), 4.72–4.18 (m, 5H, C*H*$_2$Ar, CHC*H*$_2$OCO, C*H*CH$_2$OCO), 3.89–3.66 (m, 2H, C*H* CH$_2$CH=CH$_2$, H-2), 3.81 (s, 3H, OCH_3), 3.78 (s, 6H, OCH_3), 3.47 (s, 3H, OCH_3), 3.15 (m, 3H, SC*H*$_2$CH=CH$_2$, 1H-3), 2.98 (m, 1H, 1H-3), 2.65 (m, 1H, *H*CHCH=CH$_2$), 2.48 (m, 1H, HC*H* CH=CH$_2$); MS (API-ES) m/z 697 $(M + Na)^+$.

(3*R*,6*S*)-6-(9*H* -Fluoren-9-ylmethoxycarbonylamino)-5-oxo-4-(2,4,6-trimethoxybenzyl)-3,4,5,6,7,10-hexahydro-2*H* -[1,4]thiaze-cine-3-carboxylic acid methyl ester (35)—(**Procedure E**) Compound **35** (30 mg, 71%) was obtained from **34** (44 mg, 0.07 mmol) and second generation Grubbs catalyst (17 mg, 0.02 mmol) in CH_2Cl_2 (65 mL). The product was purified by preparative thin-layer chromatography (petroleum ether–ethyl acetate, 2: 1). ^{1}H NMR ($CDCl_3$) δ 7.77 (d, J = 7.2 Hz, 2H, Ar–H), 7.63 (m, 2H, Ar–H), 7.40 (t, J = 7.4 Hz, 2H, Ar–H), 7.31 (t, J = 7.2 Hz, 2H, Ar–H), 6.16 (d, J = 6.9 Hz, 1H, NH), 6.09 (m, 2H, Ar–H), 5.65 (m, 2H, H-8, H-9), 5.36 (m, 1H, H-6), 4.70 (m, 2H, C*H*$_2$Ar), 4.43 (m, 2H, CHC*H*$_2$OCO), 4.24 (t, J = 7.2 Hz, 1H, C*H*CH$_2$OCO), 3.84–3.62 (m, 1H, H-3), 3.80 (s, 3H, OCH_3), 3.77 (s, 6H, OCH_3), 3.52–3.27 (m, 2H, H-10), 3.36 (s, 3H, OCH_3), 3.05–2.65 (m, 3H, H-2, 1H-7), 2.43 (m, 1H, 1H-7); MS (API-ES) m/z 669 $(M + Na)^+$.

(1*R*,8*S*)-11-(2,4,6-Trimethoxybenzyl)-3-thia-9,11-diazabicyclo-[6.2.2]dodec-5-ene-10,12-dione (36)—(**Procedure F**) Compound **36** (9 mg, 60%) was obtained from **35** (25 mg, 0.04 mmol) and 3.75 mL of CH_2Cl_2–piperidine (4: 1). The product was purified by preparative thin-layer chromatography (CH_2Cl_2–MeOH, 96: 4). ^{1}H NMR ($CDCl_3$) δ 6.09 (s, 3H, Ar–H, NH), 5.71 (m, 2H, H-5, H-6), 5.39 (d, J = 13.8 Hz, 1H, *H*CHAr), 4.41 (d, J = 13.8 Hz, 1H, HC*H*Ar), 4.25 (m, 1H, H-8), 4.07 (m, 1H, H-1), 3.80 (s, 3H, OCH_3), 3.78 (s, 6H, OCH_3), 3.45 (m, 1H, 1H-4), 3.26–3.01 (m, 4H, H-2, 1H-4, 1H-7), 2.54 (m, 1H, 1H-7); ^{13}C NMR ($CDCl_3$) δ 169.6, 166.4, 161.8, 160.6, 130.7, 127.5, 103.2, 90.7, 60.1, 56.0, 55.6, 55.3, 55.2, 38.2, 32.9, 30.6, 29.5; HRMS m/z found 393.1495 $(M + H)^+$. $C_{19}H_{25}N_2O_5S$ requires 393.1484; HPLC (System A) 9.7 min (99%), (System B) 11.3 min (99%).

(3*S*,6*S*)-3-Allyl-6-allyloxymethyl-1-(2,4-dimethoxybenzyl)-piperazine-2,5-dione (37)—(**Procedure F**) Compound **37** (52 mg, 42%) was obtained from **18** (206 mg, 0.33 mmol) and 10 mL of CH_2Cl_2–piperidine (4: 1). The product was purified by column chromatography on silica gel (petroleum ether–ethyl acetate, 1: 2). ^{1}H NMR ($CDCl_3$) δ 7.24 (d, J = 8.2 Hz, 1H, Ar–H), 6.45 (m, 2H, Ar–H), 6.19 (br s, 1H, NH), 5.82 (m, 2H, C*H*=CH_2), 5.22 (m, 4H, CH=C*H*$_2$), 5.10 (d, J = 14.7 Hz, 1H, *H*CHAr), 4.12 (d, J = 14.7 Hz, 1H, HC*H*Ar), 4.01 (m, 1H, H-6), 3.98 (m, 2H, OC*H*$_2$CH=CH_2), 3.93 (m, 1H, H-3), 3.82 (m, 2H, CHC*H*$_2$O), 3.79 (2s, 6H, OCH_3), 2.90 (m, 1H, *H*CHCH=CH_2), 2.60 (m, 1H, HC*H*CH=CH_2); ^{13}C NMR ($CDCl_3$) δ 166.9, 166.4, 161.0, 158.8, 134.1, 133.7, 131.9, 120.1, 118.2, 116.3, 104.8, 98.6, 72.6, 67.8, 60.1, 55.6, 55.4, 41.7, 40.6; MS (FAB) m/z 375 $(M + H)^+$.

(1*S*,8*S*,11*S*,18*S*)-21,23-Bis-(2,4-dimethoxybenzyl)-3,13-dioxa-9,19,21,23-tetraazatricyclo[16.2.2.28,11]tetracosa-5,15-diene-10, 20,22,24-tetraone (38)—(**Procedure E**) To a solution of compound **37** (15 mg, 0.04 mmol) in CH_2Cl_2 (1 mL) was added second generation Grubbs catalyst (5 mg, 0.006 mmol). The reaction mixture was stirred at room temperature for 16 h. The solvent was removed *in vacuo*, and the residue was purified by preparative thin-layer chromatography ($CHCl_3$–MeOH, 97: 3) to afford **38** (11 mg, 79%). ^{1}H NMR ($CDCl_3$) δ 9.18 (m, 2H, NH), 7.24 (d, J = 8.3 Hz, 2H, Ar–H), 6.45 (m, 4H, Ar–H), 5.65 (m, 4H, H-5, H-6, H-15, H-16), 5.18 (d, J = 14.5 Hz, 2H, *H*CHAr), 4.33 (m, 2H, 1H-4, 1H-14), 4.25 (m, 2H, 1H-2, 1H-12), 4.11 (d, J = 14.5 Hz, 2H, HC*H*Ar), 3.99 (m, 2H, H-8, H-18), 3.93 (m, 2H, H-1, H-11), 3.79 (s, 6H, OCH_3), 3.78 (s, 6H, OCH_3), 3.76 (m, 2H, 1H-2, 1H-12), 3.67 (m, 2H, 1H-4, 1H-14), 2.90 (m, 2H, 1H-7, 1H-17), 2.68 (m, 2H, 1H-7, 1H-17); ^{13}C NMR ($CDCl_3$) δ 169.5, 166.3, 161.0, 158.8, 131.8, 130.6, 127.9, 116.2, 104.9, 98.6, 72.5, 69.8, 60.5, 55.6, 55.3, 40.8, 40.0; HRMS m/z found 693.3149 $(M + H)^+$. $C_{36}H_{45}N_4O_{10}$ requires 693.3136.

Pharmacological analyses

Cell culture and membrane preparation—Human 1321N1 astrocytoma cells transfected individually with the h$P2Y_{1,2,4,6,11}$ receptors[26,33,40] were grown at 37 °C in a humidified incubator with 5% CO_2–95% air in Dulbecco's modified Eagle's medium (JRH Biosciences, Inc.) supplemented with 10% fetal bovine serum (FBS), 100 Units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 2 mM L-glutamine. The cells were grown to *ca.* 60% confluence for the experiments.

For membrane preparation, human astrocytoma cells expressing human $P2Y_1$ receptors were grown to approximately 80% confluence and then harvested. The cells were homogenized and suspended and then centrifuged at 100 *g* for 5 min at room temperature. The pellet was resuspended in 50 mM tris(hydroxymethyl)aminomethane (Tris) hydrochloride buffer (pH 7.4). The suspension was homogenized with a Polytron homogenizer (Brinkmann) for 10 s

and was then recentrifuged at 20 000 *g* for 20 min at 4 °C. The resultant pellets were resuspended in Tris buffer (pH 7.4), and the suspension was stored at −80 °C until the binding experiments. The protein concentration was measured with the Bradford assay.[44]

Storage of test substances—Agonists were dissolved as stock solutions in Tris buffer (pH 7.4), and the DKP derivatives were stored as frozen stock solutions in DMSO (5 mM) at −20 °C. Prior to use the DMSO solutions were warmed briefly to 50–60 °C.

Determination of inositol phosphates—The quantity of inositol phosphates was measured essentially as reported.[25,26,33,41] The $P2Y_{1,2,4,6,11}$-1321N1 cells were grown to confluence in 6-well plates in the presence of *myo*-[^{3}H]inositol (2 μCi mL^{-1}) for 24 h. Cells were then treated for 30 min at 37 °C with antagonists or buffer in the presence of 20 mM LiCl, followed by another 30 min of incubation at 37 °C with the appropriate agonist. Agonists used were: $P2Y_1$, 2-MeSADP; $hP2Y_2$, UTP; $hP2Y_4$, UTP; $hP2Y_6$, UDP; $hP2Y_{11}$, ATP. The reaction was terminated upon aspiration of the medium and addition of cold formic acid (20 mM). After 30 min, supernatants were neutralized with NH_4OH, and applied to Bio-Rad Dowex AG 1-X8 anion exchange columns. All of the columns were then washed with water followed by a 60 mM sodium formate solution containing 5 mM sodium tetraborate. Total inositol phosphates were eluted with 1 M ammonium formate containing 0.1 M formic acid, and radioactivity values were measured using a liquid scintillation counter.

Radioligand binding assay—$P2Y_1$ receptor binding experiments were performed as previously described.[39] Briefly, membranes (40 μg protein) from astrocytoma cells stably expressing human $P2Y_1$ receptors were incubated with [^{3}H]MRS2279 (8 nM) for 30 min at 4 °C in a total assay volume of 200 μL. For adenosine A_1 receptor binding, an agonist radioligand [^{3}H]R-PIA (2.0 nM) was incubated with membranes (40 lg protein per tube) from CHO cells stably expressing human adenosine A_1 receptors for 60 min at 25 °C.[40] Radiolabeled ligand concentrations used in all assays approximated the K_d values of the receptor. Binding reactions were terminated by filtration through Whatman GF/B glass-fiber filters under reduced pressure with an MT-24 cell harvester (Brandel), and radioactivity was determined with a 1414 liquid scintillation counter (Wallac, Win Spectral, Perkin Elmer Life Sciences).

Calcium mobilization assay

Human astrocytoma cells stably expressing human $P2Y_1$ receptors were cultured in Dulbecco's modified Eagle's medium (DMEM, JRH Biosciences, Inc., Lenexa, KS, USA) and F12 (1: 1) supplemented with 10% fetal bovine serum, 100 units penicillin mL^{-1}, 100 μg streptomycin mL^{-1}, 2 μmol glutamine mL^{-1}, and 500 μg geneticin mL^{-1}. For the assay, cells were grown overnight in 100 μL of media in 96-well flat-bottom plates at 37°C at 5% CO_2 or until approximately 60–80% confluency. The calcium assay kit (Molecular Devices, Sunnyvale, CA) was used as directed without washing of the cells, and with probenecid added to the loading dye at a final concentration of 2.5 mM to increase dye retention. Cells were loaded with 50 μL of dye with probenecid to each well and incubated for 45 min at room temperature. The compound plate was prepared using dilutions of various compounds in Hank's buffer. For antagonist studies, both agonist and antagonist were added to the sample plate. Samples were run in duplicate using a Molecular Devices Flexstation I at room temperature. Cell fluorescence (excitation = 485 nm, emission = 525 nm) was monitored following exposure to the compound. Increases in intracellular calcium are reported as the maximum fluorescence value after exposure minus the basal fluorescence value before exposure.

Data analysis

IC_{50} values obtained in radioligand binding assays and in assays of inhibition of agonist-stimulated inositol phosphate accumulation were calculated by a four-parameter logistic equation and the GraphPad software package (GraphPad, San Diego, CA). All concentration–effect curves were repeated in at least three separate experiments, carried out in duplicate or triplicate with different membrane preparations or different 1321N1 cell cultures.

Molecular modeling

Molecular modeling was carried out with the module Discover3 of InsightII (Accelrys, Inc.) on Silicon Graphics Origin 200 computer, using the CFF91 forcefield.[45]

An NVT (constant-volume/constant-temperature) molecular dynamics simulation was carried out for 500 ns at 298 K, with a time step of 1 fs. Conformations were sampled at regular intervals of 100 ns and energy minimized employing the BFGS Newton method, until an RMS gradient lower than 0.00001 kcal mol^{-1} $Å^{-1}$ was reached.

For a description of the puckering of the DKP ring we used the coordinates Θ (phase angle of symmetrical interconversion), P_2 (phase angle of pseudorotation), and Q (total puckering amplitude) as defined by Haasnoot,[46] utilizing the final formulae that we recently reported.[40]

Supplemental Material

Refer to Web version on PubMed Central for supplementary material.

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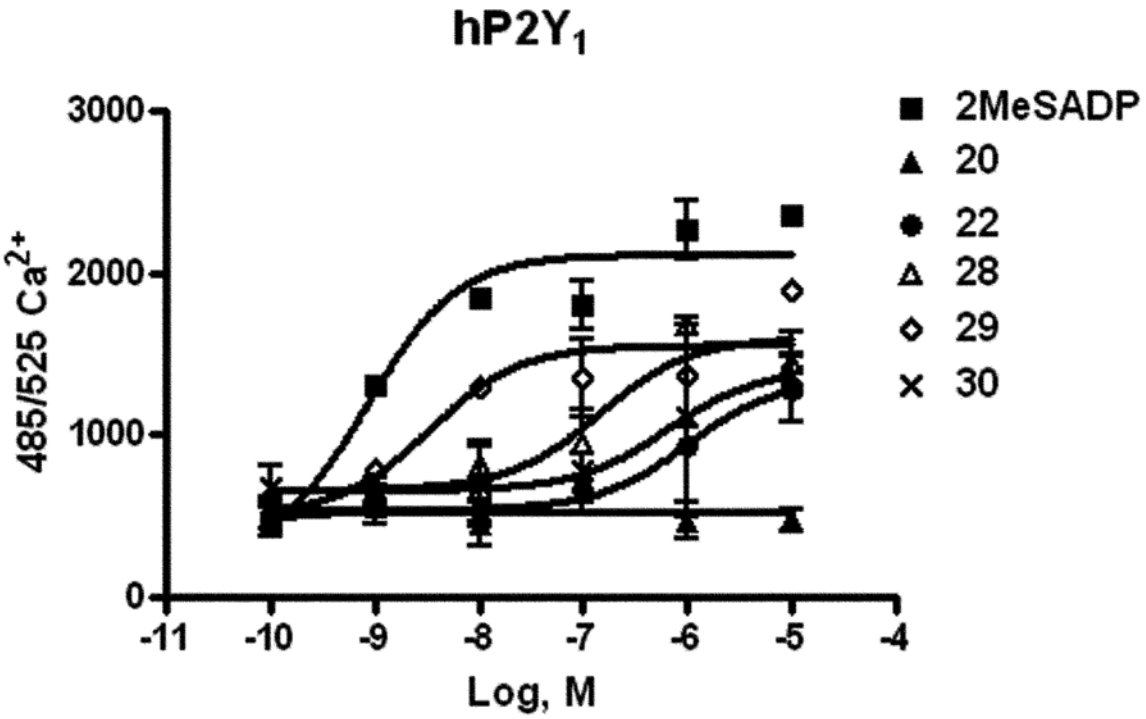


Fig. 1.

Effects of DKP derivatives (10 μM, structures in Scheme 2) on concentration–response curves for intracellular Ca^{2+} changes induced by 2-MeSADP acting at $P2Y_1$ receptors expressed in 1321N1 astrocytoma cells. The cells were pre-treated for 20 min at room temperature with antagonist before application of the agonist 2-MeSADP.

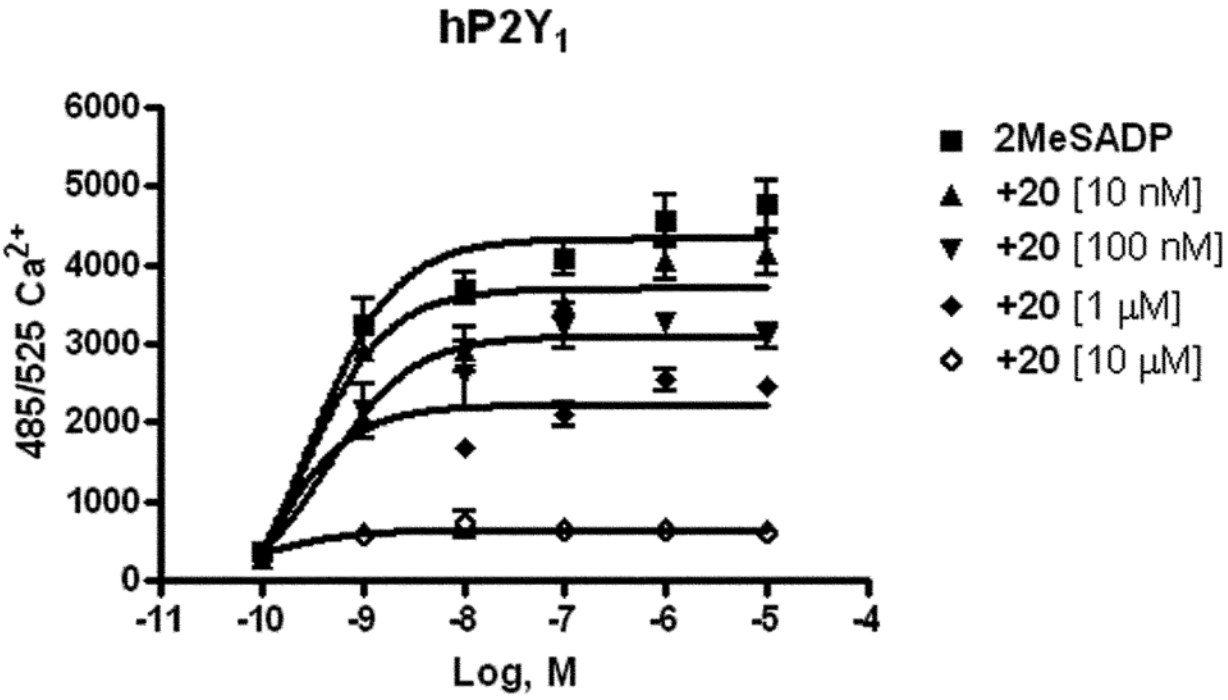
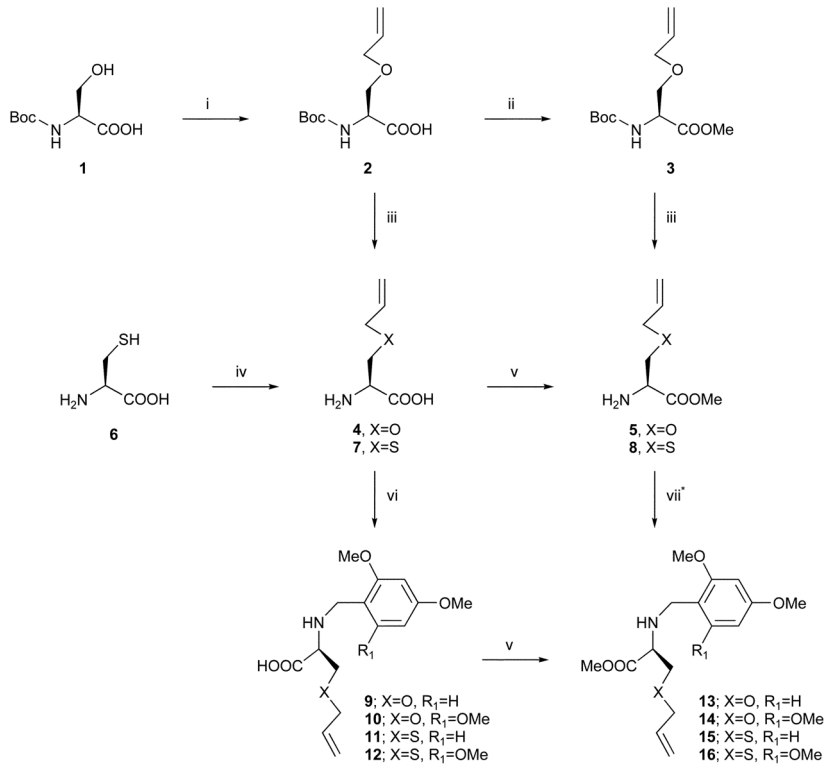


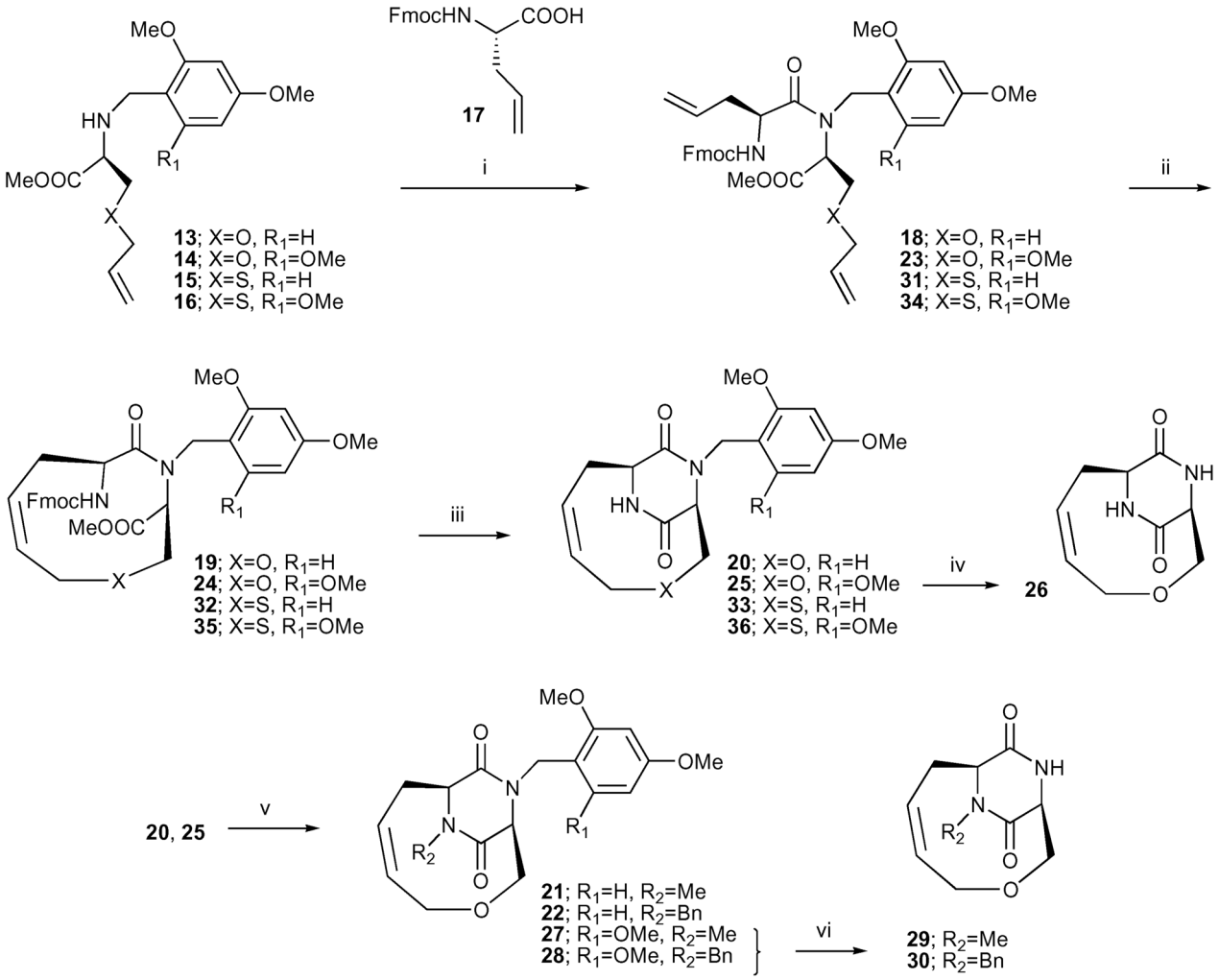
Fig. 2.

Effect of compound **20** on the concentration–response curve of the $[Ca^{2+}]i$ transient induced by 2MeSADP in $P2Y_1$ receptors expressed in 1321N1 astrocytoma cells. The cells were pre-treated for 20 min at room temperature with different concentrations of compound **20** before application of the agonist 2-MeSADP.



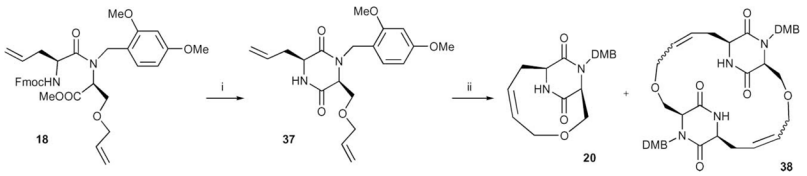
Scheme 1.

Preparation of protected Ser and Cys derivatives. *Reagents and conditions*: (i) NaH, allylbromide, DMF, rt, 94%; (ii) $TMSCHN_2$, Et_2O–MeOH 1: 1, rt, 78%; (iii) TFA, CH_2Cl_2, rt, 93% (**5**); (iv) EtONa, allylbromide, EtOH, rt, 76%; (v) $TMSCHN_2$, Et_2O–CH_2Cl_2 – MeOH 1: 5: 5, rt, 33% (**13**), 32% (**14**), 55% (**15**), 22% (**16**); (vi) 2,4-dimethoxybenzaldehyde or 2,4,6-trimethoxybenzaldehyde, $NaBH_3CN$, MeOH, rt, 53% (**9**), 53% (**10**), 41% (**11**), 41% (**12**); (vii) 2,4-dimethoxybenzaldehyde or 2,4,6-trimethoxybenzaldehyde, $NaBH(OAc)_3$, CH_2Cl_2, rt, 85% (**13**), 82% (**14**), 74% (**15**), 58% (**16**), *partial racemization was obtained in this reaction.



Scheme 2.

Synthesis of bicyclic DKP derivatives. The RCM reaction preceded the closure of the DKP ring. *Reagents and conditions*: (i) HATU, HOAt, NEM, CH_2Cl_2 –DMF 9: 1, rt, 53% (**18**), 40% (**23**), 37% (**31**), 41% (**34**); (ii) second generation Grubbs catalyst, CH_2Cl_2, rt, 60% (**19**), 57% (**24**), 57% (**32**), 71% (**35**); (iii) piperidine–CH_2Cl_2 1: 4, rt, 93% (**20**), 73% (**25**), 73% (**33**), 60% (**36**); (iv) CH_2Cl_2 –TFA 2: 1, rt, 96%; (v) MeI or BnBr, NaH, THF, rt, 63% (**21**), 60% (**22**), 96% (**27**), 85% (**28**); (vi) CH_2Cl_2 –TFA 2: 1, rt, 63% (**29**), 89% (**30**).



Scheme 3.

Synthesis of bicyclic DKP derivatives. The closure of the DKP ring preceded the RCM reaction. DMB = 2,4-$(MeO)_2$ $PhCH_2$. *Reagents and conditions*: (i) piperidine–CH_2 Cl_2 1: 4, rt, 42%; (ii) **37** (0.2 mM), second generation Grubbs catalyst, CH_2 Cl_2, rt, 21% (**20**), 43% (**38**); **37** (40 mM), 79% (**38**).