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PAPER

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PAPER

An efficient synthesis of benzodiazepinyl phosphonates as clostripain inhibitors *via* $FeCl_3$ catalyzed four-component reaction†

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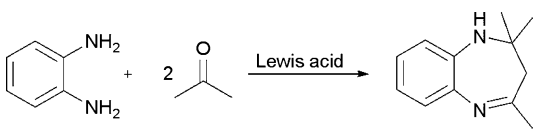
A novel one-pot route for the synthesis of benzodiazepinyl phosphonates (BDPs) has been achieved. $FeCl_3$ efficiently catalyzed four-component condensation of diamines, acetone and phosphites in the presence of molecular sieves to furnish BDPs as novel chemical entities with good yield. The synthesized BDPs have shown significant protease inhibition activity against clostripain, a disease model for gas gangrene, suggesting that these novel chemical entities could be further explored as cysteine protease inhibitors.

Introduction

In recent years, multicomponent reactions (MCRs) have gained tremendous attention of medicinal as well as organic chemists for the generation of compound libraries of novel chemical entities to satisfy the need of high-throughput screening for new bioactive molecules having diversified scaffolds. In MCR, more than two reactants are reacted in a reaction flask to furnish a product that incorporates substantial portions of all the components.[1,2] In its true form, MCR involves formation of several bonds in a single operation without the need for isolation of the intermediates formed, changing the reaction conditions or adding further reagents. Many important named reactions are MCR in nature,[3] *e.g.* Strecker, Hantzsch, Biginelli, Mannich, Passirini, Ugi reactions, *etc.* Some classes of compounds such as isonitriles and 1,3-dicarbonyl compounds have found wide applications in a variety of MCRs. Similarly alkyl/aryl phosphites have also been utilized as an important participating component in some MCRs.[4]

The benzodiazepines represent a biologically active class of compounds which exhibits a wide range of therapeutic and pharmacological properties[5] such as anticonvulsant, anti-anxiety, analgesic, hypnotic, sedative, antidepressant, anti-inflammatory, inhibition of hepatitis C NS5B RNA polymerase,[6] antagonism of platelet-activating factor, psychotropic activity, caspase-1 inhibitors,[7] antitumor agents[5] and β-secretase inhibition.[5] α-Aminophosphonates have also shown various biological activities such as peptide mimics,[8] haptens of catalytic antibodies,[9] antibiotics and pharmacological agents,[10] and herbicides.[11] Therefore, we opined that benzodiazepinyl phosphonates (BDPs) will be an interesting class of compounds as it combines these two biologically active moieties and also the synthesis of benzodiazepinyl phosphonates could be achieved in one pot utilizing MCR. Further, α-aminophosphonates are considered to be the structural analogues of the corresponding α-amino acids and transition-state mimics of peptide hydrolysis, the phosphonate group of α-aminophosphonates can act as an electrophile which is the common requirement of cysteine protease inhibitors.[12] This generates the possibility that BDPs can act as a cysteine protease inhibitor. Clostripain is one of the cysteine proteases associated with collagenase, isolated from *Clostridium histolyticum*, an anaerobic rod-shaped, spore-forming bacillus, which belongs to the group of *Clostridium* spp,[13] and causes deadly gas gangrene, a severe pathological condition. These *Clostridium* species are also responsible for various disorders like pseudomembranous colitis, food poisoning, tetanus and enteroxemia. Therefore, inhibitors of clostripain could be utilized in the therapy of gas gangrene.

Recently, we have described the three-component reaction of aldehydes, amines, and diethyl phosphite catalyzed by Amberlite IR 120 (acidic)[14a] or bismuth nitrate[14b] affording the corresponding α-amino phosphonates in excellent yields. In the literature, syntheses of benzodiazepines have been accomplished by reacting *o*-phenylenediamine and ketones catalyzed by various Lewis acids (Scheme 1).[15] We envisaged that further nucleophilic attack of phosphite on the imine would result in the formation of BDPs in one pot in a true MCR fashion (Scheme 2).



Scheme 1 Reported one pot synthesis of benzodiazepine.

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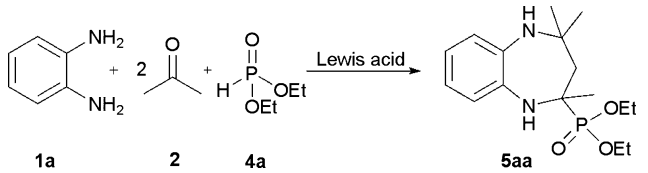
† Electronic supplementary information (ESI) available: Full experimental procedures and copies of NMR spectra of all the compounds. See DOI: 10.1039/c0ob01102a

‡ Deceased.



Scheme 2 Proposed one-pot synthesis of benzodiazepinyl phosphonates.

Results and discussion

Chemistry

Initially the reaction of *o*-phenylenediamine **1a**, acetone **2** and diethylphosphite **4a** was carried out in the presence of silica–perchloric acid; however, BDP **5aa** was obtained in low yield (12%) even after 2 days. The slow reaction and low yield could be attributed to the less electrophilic ketimine which results in the slow attack of the phosphite. To overcome this problem, we screened several catalysts and reaction conditions as shown in Table 1.

Catalyst screening revealed that $FeCl_3$ (10 mol%) was the best catalyst furnishing the product in 43% yield; however, the reaction was slow (entry 7, Table 1). To circumvent this problem, reflux or microwave irradiation conditions were employed which resulted in decomposition (entries 8 and 9, respectively). Since the reaction proceeds with the initial formation of the imine with concomitant generation of water which can decompose or deactivate the catalyst,[14] we opined that trapping of the water by the use of molecular sieves would be beneficial. When the reaction was carried out in the presence of preactivated molecular sieves (4 Å) at room temperature, the reaction was complete within 1 h with 63% yield of the product (entry 10). After optimizing the reaction conditions, we carried out generalization of this reaction by reacting structurally diverse diamines, ketone and phosphites. The results are summarized in Table 2.

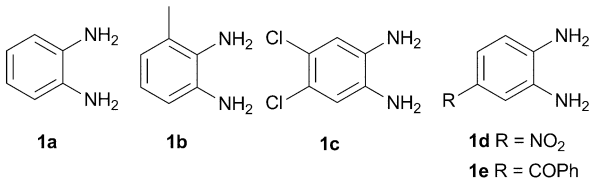
Different substituted diamines underwent one-pot reaction to yield corresponding benzodiazepinyl phosphonates (Fig. 1). In the case of substituted benzene-1,2-diamine, two regioisomers were formed, however, which could not be separated by repeated chromatography (Scheme 3). Thus, the ratio of the regioisomers was calculated from ^{1}H NMR, *e.g.* in the case of 3-methyl-1,2-diamine **1b**, two regioisomers **5ba** and **5ba′** were formed in the ratio of 1 : 3 (see the Experimental section).

Table 1 Catalyst screening and reaction condition optimization for one-pot synthesis of benzodiazepinyl phosphonate

Sl. no.	Catalyst[a,b]	Conditions	Time	Yield (%)
1	$HClO_4$–SiO_2[16]	RT	2 d	12
2	$TaCl_5$–SiO_2[17]	RT	2 d	23
3	$Mg(ClO_4)_2$	RT	2 d	15
4	$AlCl_3$	RT	2 d	15
5	$InCl_3$	RT	2 d	25
6	$BiCl_3$	RT	2 d	10
7	$FeCl_3$	RT	2 d	43
8	$FeCl_3$	Reflux	6 h	Decomposition
9	$FeCl_3$	Microwave	1 min	Decomposition
10	$FeCl_3$	RT, MS 4 Å	1 h	63

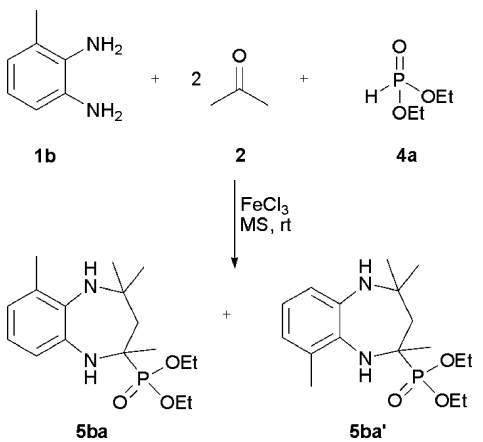
[a] For entries 1–2, 10 wt.% of the catalyst was used. [b] For entries 3–10, 10 mol% of the catalyst was used.

Table 2 One-pot synthesis of benzodiazepinyl phosphonate catalyzed by $FeCl_3$



	$HPO(OEt)_2$ 4a	$HPO(OBu)_2$ 4b	$HPO(OAllyl)_2$ 4c	$HPO(OMe)_2$ 4d		
Entry	Amine	Phosphite	Product	Yield (%)[a]	Ratio of isomers[b]	
1	**1a**	**4a**	**5aa**	63	—	
2	**1a**	**4b**	**5ab**	63	—	
3	**1a**	**4c**	**5ac**	66	—	
4	**1a**	**4d**	**5ad**	65	—	
5	**1b**	**4a**	**5ba+5ba′**	42	1 : 6	
6	**1b**	**4b**	**5bb+5bb′**	50	1 : 8	
7	**1b**	**4c**	**5bc+5bc′**	52	1 : 5	
8	**1c**	**4a**	**5ca**	59	—	
9	**1c**	**4b**	**5cb**	55	—	
10	**1c**	**4c**	**5cc**	52	—	
11	**1c**	**4d**	**5cd**	51	—	
12	**1d**	**4a**	**5da + 5da′**	63	1 : 8	
13	**1d**	**4b**	**5db + 5db′**	59	0 : 1	
14	**1d**	**4c**	**5dc + 5dc′**	57	0 : 1	
15	**1d**	**4d**	**5dd + 5dd′**	53	1 : 1	
16	**1e**	**4a**	**5ea + 5ea′**	43	3 : 4	
17	**1e**	**4b**	**5eb + 5eb′**	42	1 : 3	
18	**1e**	**4c**	**5ec + 5ec′**	50	1 : 2	
19	**1e**	**4d**	**5ed + 5ed′**	49	1 : 1	

[a] Yields refer to the isolated yields. [b] Ratio of regioisomers formed are calculated based on ^{1}H NMR spectra.



Scheme 3 One-pot synthesis of benzodiazepinyl phosphonate from 2,3-diamino toluene.

In the case of the reaction of butylphosphite and allylphosphite with nitro-substituted diamine (entries 13 and 14, respectively) exclusively one regioisomer was formed. The characterization of the regioisomers was based on HMBC correlation as shown in Fig. 2. In compound **5db′**, the aromatic proton at δ 7.67 (dd, 1H) showed HMBC correlation with the carbon at δ 139.0, which in turn did not show any C–P coupling, suggesting a *syn*-structure for the regioisomer **5db′**. Similarly, in the case of **5dc′**, the proton at δ 7.66 (dd, 1H) showed HMBC correlation with the carbon at

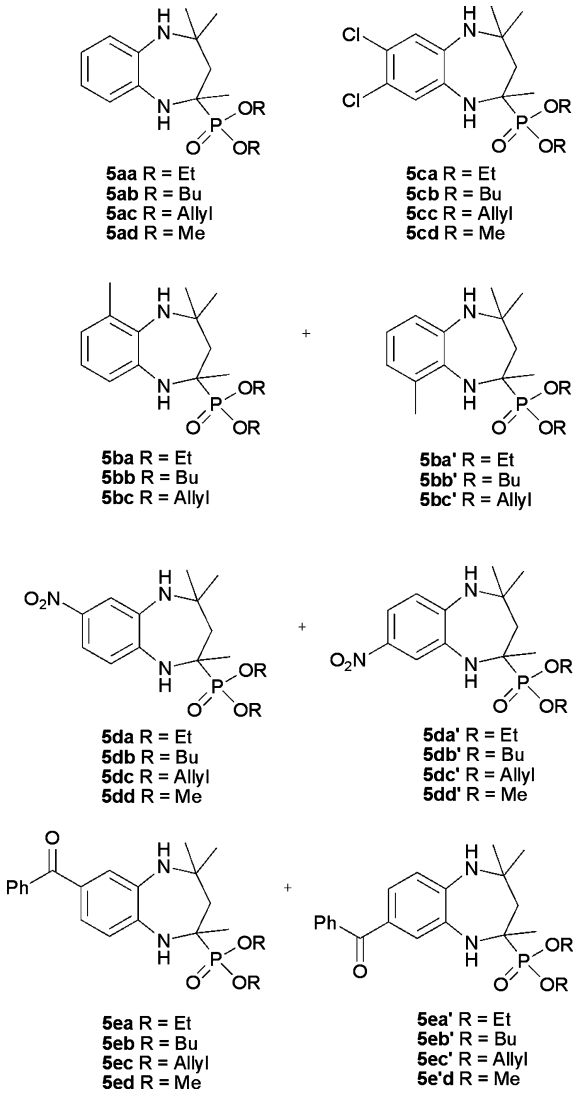


Fig. 1 Structures of benzodiazepinyl phosphonates synthesized through one-pot reaction.

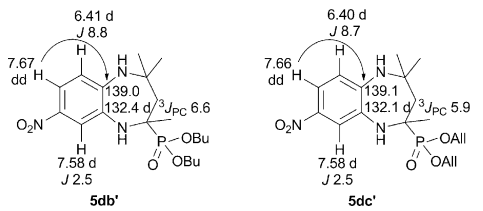
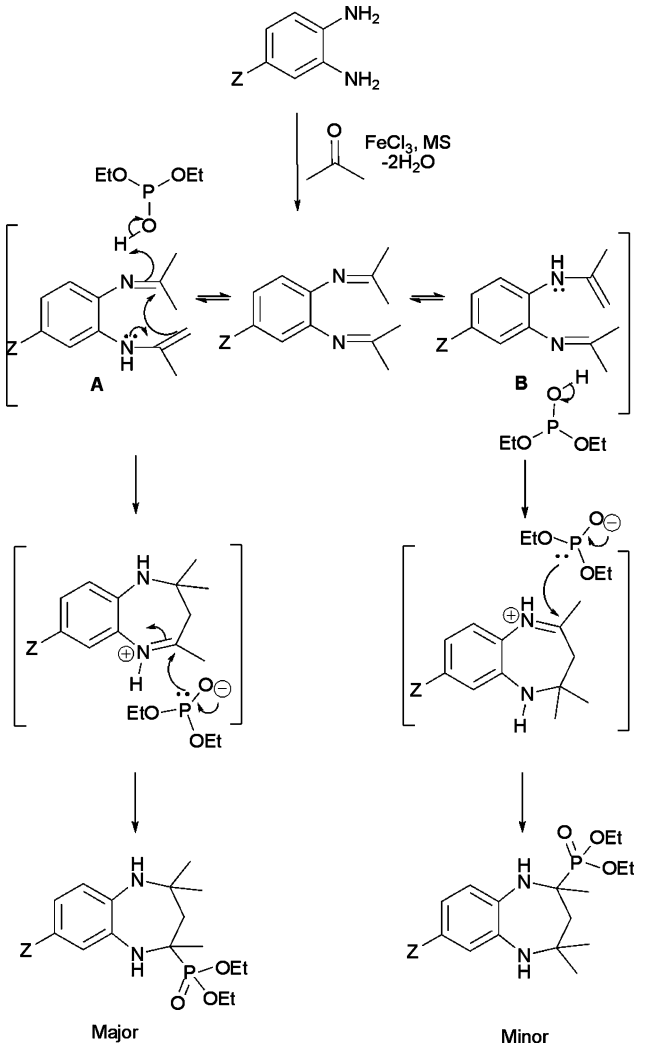


Fig. 2 HMBC correlations of **5db′** and **5dc′**.

δ 139.1, which also did not show any C–P coupling, suggesting a *syn*-structure for **5dc′**. The above results indicate that in the case of unsymmetrical diamines, the major product formed is the *syn*-regioisomer. Further, this could be explained on the basis of the plausible reaction mechanism shown in the Scheme 4. Initially, di-imine is formed by the reaction of ketone with diamine catalyzed by $FeCl_3$. The di-imine exists in two tautomeric forms, A and B. The tautomeric form A is more reactive than B since the lone pair of electrons on the nitrogen in B is conjugated with the electron-withdrawing group Z and therefore not easily available for donation while the lone pair of electrons on nitrogen in A



Scheme 4 Plausible mechanism for the formation of benzodiazepinyl phosphonates (major and minor regioisomers).

is not conjugated with the electron-withdrawing Z group, and thereby easily donated leading to the intermediate that gives the *syn*-regioisomer as the major product. Similarly, the reaction of the tautomeric form B gives the *anti*-regioisomer as the minor product.

Several ketones such as cyclobutanone, ethyl methyl ketone, isopropyl methyl ketone and acetone were employed; however, only acetone furnished the corresponding products, BDPs. In the case of the other ketones, only intermediate ketimines were formed. This could be due to the steric factor. With higher ketones, the ketimines formed are so sterically crowded that it could not allow phosphite to attack even if the reaction was continued for a prolonged period of time. All the phosphites employed worked well except triphenyl phosphite and diphenyl phosphite. This can also be explained on the basis of steric factors.

The synthesized benzodiazepinyl phosphonates were tested for their *in vitro* inhibition of clostripain and the results are summarized in Table 3. Compound **5ba + 5ba′** derived from 2,3-diamino toluene and diethyl phosphite inhibited the clostripain enzyme with an IC_{50} value of 32 μM. When the phosphite was changed to allyl (**5bc + 5bc′**) and butyl (**5bb + 5bb′**), the activity dropped to

Table 3 Inhibition activity of synthesized BDPs against clostripain

Entry	Compound code	IC_{50} values/μM	Entry	Compound code	IC_{50} values/μM
1	**5aa**	ND	11	**5cd**	150
2	**5ab**	780	12	**5da + 5da′**	>300
3	**5ac**	>300	13	**5db + 5db′**	ND
4	**5ad**	490	14	**5dc + 5dc′**	165
5	**5ba + 5ba′**	32	15	**5dd + 5dd′**	220
6	**5bb + 5bb′**	278	16	**5ea + 5ea′**	36
7	**5bc + 5bc′**	80	17	**5eb + 5eb′**	140
8	**5ca**	>300	18	**5ec + 5ec′**	70
9	**5cb**	175	19	**5ed + 5ed′**	90
10	**5cc**	>300			

ND = Not determined.

80 and 278 μM, respectively. A similar trend in the activity profile was observed in the case of benzodiazepinyl phosphonate obtained by the reaction of 3,4-diamino benzophenone. Compound **5ea + 5ea′** showed an IC_{50} value of 36 μM, which dropped to 70 and 140 μM in the case of **5ec + 5ec′** and **5eb + 5eb′**, respectively. The benzodiazepinyl phosphonates derived from symmetrical diamines (**1a** and **1c**) were found to be comparatively less potent than their corresponding benzodiazepinyl phosphonates derived from unsymmetrical diamines (**1b**, **1d** and **1e**).

Conclusions

In summary, a four-component reaction of diamines, ketones and phosphite catalyzed by $FeCl_3$ has been established to generate novel chemical entities, benzodiazepinyl phosphonates (BDPs). All the synthesised compounds were assayed *in vitro* for their efficacies against clostripain, a disease model for gas gangrene. Some of the synthesized BDPs showed remarkable cysteine protease inhibition activities in the micromolar range, thereby suggesting that these chemical entities could be further explored for their protease inhibition to obtain a lead compound.

Experimental section

General

The FT-IR spectra were recorded on an FT-IR-8300 Shimadzu spectrometer and microanalyses were carried out on a Carlo-Erba instrument. NMR spectra were recorded on Bruker ACF 200 and AV200 (200 MHz for ^{1}H NMR and 50 MHz for ^{13}C NMR) and AV400 (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometers, using $CDCl_3$ as solvent. Tetramethylsilane (0.00 ppm) served as an internal standard in ^{1}H NMR and $CDCl_3$ (77.0 ppm) in ^{13}C NMR, respectively. Chemical shifts are expressed in parts per million (ppm). In the case of NMR data of a mixture of regioisomers, the peaks corresponding to the major isomer are given. Mass spectra were recorded on LC-MS/MS-TOF API QSTAR PULSAR spectrometer, samples introduced by infusion method using the Electrospray Ionization Technique (ESI). Clostripain, *N*-α-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA), dithiothreitol (DTT), dimethoxy sulfoxide (DMSO) and calcium chloride ($CaCl_2$) were purchased from Sigma Chem. Co. (USA). All other chemicals were of analytical grade.

Typical experimental procedure. To a mixture of *o*-phenylenediamine (1 mmol), acetone (0.5 mL) and molecular sieves (4 Å, 50 mg), $FeCl_3$ (10 mol%) and phosphite (1 mmol) were added. The reaction mixture was stirred at room temperature for 1 h. After completion of the reaction (TLC), saturated aq. $NaHCO_3$ (10 mL) was added to the reaction mixture and the product was extracted with EtOAc (3 × 20 mL). The combined organic layer was washed with brine (30 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum to furnish the crude product which was purified by silica gel column chromatography (ethyl acetate : pet. ether, 40–60%).

*Diethyl-2,4,4-trimethyl-2,3,4,5-tetrahydro-1*H*-benzo-[*b*][1,4]-diazepin-2-ylphosphonate (**5aa**).* Pale yellow syrup; (Found: C, 58.95; H, 8.28; N, 8.63. Calc. for $C_{16}H_{27}N_2O_3P$: C, 58.88; H, 8.34; N, 8.58%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3420, 3367, 3019, 1604, 1518, 1424, 1216, 1048 and 1031; δ_H (200 MHz; $CDCl_3$) 1.29-1.36 (12 H, m), 1.56 (3 H, d, $^3J_{PH}$ 17.2), 1.72–1.82 (1 H, m), 2.14–2.37 (1 H, m), 4.08–4.24 (4 H, m) and 6.62–6.81 (4 H, m); δ_C (50 MHz, $CDCl_3$) 16.5 (d, $^3J_{PC}$ 5.9), 16.6 (d, $^3J_{PC}$ 5.5), 23.5, 30.5, 33.6, 44.0 (d, $^2J_{PC}$ 2.6), 53.2 (d, $^3J_{PC}$ 14.6), 56.2 (d, $^1J_{PC}$ 148.2), 62.5 (d, $^2J_{PC}$ 7.7), 63.2 (d, $^2J_{PC}$ 7.0), 121.6, 121.8, 121.9, 122.0, 136.8 (d, $^3J_{PC}$ 12.4) and 137.5; δ_P (161 MHz, $CDCl_3$) 29.1; *m/z* (ESI): 327.29 $(M + H)^+$, 349.28 $(M + Na)^+$.

*Dibutyl-2,4,4-trimethyl-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]-diazepin-2-ylphosphonate (**5ab**).* Yellow syrup; (Found: C, 62.78; H, 9.34; N, 7.41. Calc. for $C_{20}H_{35}N_2O_3P$: C, 62.80; H, 9.22; N, 7.32%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3421, 3020, 2972, 1599, 1476, 1423, 1216 and 1030; δ_H (400 MHz, $CDCl_3$) 0.91 (6 H, t, *J* 7.3), 1.28 (3 H, s), 1.30 (3 H, s), 1.56 (3 H, d, $^3J_{PH}$ 17.2), 1.26–1.66 (8 H, m), 1.72–1.77 (1 H, m), 2.15–2.21 (1 H, m), 4.04–4.14 (4 H, m) and 6.60–6.76 (4 H, m); δ_C (100 MHz, $CDCl_3$) 13.6, 18.7, 23.6, 30.6, 32.6 (d, $^3J_{PC}$ 5.8), 32.7 (d, $^3J_{PC}$ 5.5), 33.4, 43.9 (d, $^2J_{PC}$ 2.6), 53.2 (d, $^3J_{PC}$ 14.6), 56.4 (d, $^1J_{PC}$ 147.8), 66.2 (d, $^2J_{PC}$ 8.0), 66.8 (d, $^2J_{PC}$ 7.3), 121.5, 121.6, 121.8, 121.9, 136.8 (d, $^3J_{PC}$ 11.7) and 137.4; δ_P (161 MHz, $CDCl_3$) 29.77; *m/z* (ESI) 383.32 $(M + H)^+$, 405.31 $(M + Na)^+$.

*Diallyl-2,4,4-trimethyl-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]-diazepin-2-ylphosphonate (**5ac**).* Yellow syrup; (Found: C, 61.76; H, 7.69; N, 7.92. Calc. for $C_{18}H_{27}N_2O_3P$: C, 61.70; H, 7.77; N, 7.99%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3420, 3019, 2934, 1602, 1522, 1424, 1216 and 1045; δ_H (200 MHz, $CDCl_3$) 1.30 (3 H, s), 1.33 (3 H, s), 1.59 (3 H, d, $^3J_{PH}$ 17.4), 1.74–1.84 (1 H, m), 2.18–2.31 (1 H, m), 4.52–4.65 (4 H, m), 5.14–5.43 (4 H, m), 5.83–6.03 (2 H, m) and 6.61–7.01 (4 H, m); δ_C (50 MHz, $CDCl_3$) 23.7, 30.6, 33.5, 43.8 (d,

$^{2}J_{PC}$ 2.2), 53.2 (d, $^{3}J_{PC}$ 14.6), 56.6 (d, $^{1}J_{PC}$ 147.1), 66.9 (d, $^{2}J_{PC}$ 7.7), 67.4 (d, $^{2}J_{PC}$ 6.9), 118.0 (d, $^{4}J_{PC}$ 2.2), 121.5, 121.8, 121.8, 122.0, 133.0 (d, $^{3}J_{PC}$ 5.9), 133.2 (d, $^{3}J_{PC}$ 5.9), 136.5 (d, $^{3}J_{PC}$ 11.7) and 137.5; δ_P (161 MHz, $CDCl_3$): 30.43; *m/z* (ESI) 351.27 $(M + H)^+$, 373.25 $(M + Na)^+$.

*Dimethyl-2,4,4-trimethyl-2,3,4,5-tetrahydro-1*H*-benzo-[*b*]-[1,4] diazepin-2-ylphosphonate (**5ad**).* Yellow syrup; (Found: C, 56.42; H, 7.70; N, 9.42. Calc. for $C_{14}H_{23}N_2O_3P$: C, 56.37; H, 7.77; N, 9.39%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3420, 3019, 2934, 1614, 1502, 1216 and 1054; δ_H (400 MHz, $CDCl_3$) 1.30 (3 H, s), 1.31 (3 H, s), 1.56 (3 H, d, $^{3}J_{PH}$ 17.3), 1.75–1.80 (1 H, m), 2.04–2.37 (1 H, m), 3.79 (3 H, d, $^{3}J_{PH}$ 10.5), 3.81 (3 H, d, $^{3}J_{PH}$ 10.5), 6.64–6.66 (1 H, m), 6.72–6.74 (1 H, m) and 6.77–6.80 (2 H, m); δ_C (100 MHz, $CDCl_3$): 23.5, 30.3, 33.6, 44.0 (d, $^{3}J_{PC}$ 2.2), 53.1 (d, $^{3}J_{PC}$ 14.6), 53.2 (d, $^{2}J_{PC}$ 8.1), 54.3 (d, $^{2}J_{PC}$ 7.3), 56.6 (d, $^{1}J_{PC}$ 147.5), 121.6, 121.8, 121.9, 122.2, 136.6 (d, $^{3}J_{PC}$ 12.5) and 137.5; δ_P (161 MHz, $CDCl_3$) 32.27; *m/z* (ESI) 299.23 $(M + H)^+$, 321.20 $(M + Na)^+$.

*Diethyl-2,4,4,9-tetramethyl-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepin-2-ylphosphonate (**5ba + 5ba′**) (1 : 6 regio-isomeric mixture).* Yellow syrup; (Found: C, 59.83; H, 8.63; N, 8.34. Calc. for $C_{17}H_{29}N_2O_3P$: C, 59.98; H, 8.59; N, 8.23%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3420, 3367, 3019, 1599, 1476, 1421, 1216 and 1029; δ_H (500 MHz, $CDCl_3$) 1.29–1.38 (12 H, m), 1.58 (3 H, d, $^{3}J_{PH}$ 17.9), 1.77–1.81 (1 H, m), 2.13–2.16 (1 H, m), 2.23 (3 H, s), 4.13–4.23 (4 H, m), 6.55 (1 H, d, *J* 7.6), 6.66–6.69 (1 H, m) and 6.74 (1 H, d, *J* 7.6); δ_C (125 MHz, $CDCl_3$): 16.5 (d, $^{3}J_{PC}$ 5.5), 18.0, 23.4, 30.4, 33.4, 43.6 (d, $^{2}J_{PC}$ 1.8), 52.8 (d, $^{3}J_{PC}$ 15.4), 56.0 (d, $^{3}J_{PC}$ 148.0), 62.7 (d, $^{2}J_{PC}$ 8.2), 62.8 (d, $^{2}J_{PC}$ 7.3), 120.4, 120.9, 124.1, 128.2, 136.0 (d, $^{2}J_{PC}$ 10.9 and 137.3; δ_P (202 MHz, $CDCl_3$) 30.30; *m/z* (ESI) 341.57 $(M + H)^+$, 363.57 $(M + Na)^+$.

*Dibutyl-2,4,4,9-tetramethyl-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepin-2-ylphosphonate (**5bb + 5bb′**) (1 : 8 regio-isomeric mixture).* Yellow syrup; (Found: C, 63.55, H, 9.50; N, 7.10. Calc. for $C_{21}H_{37}N_2O_3P$: C, 63.61; H, 9.41; N, 7.07%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3420, 3360, 3019, 1599, 1518, 1476, 1424, 1215 and 1022; δ_H (200 MHz, $CDCl_3$): 0.88–0.98 (6 H, m), 1.25–1.73 (17 H, m), 1.76–1.83 (1 H, m), 2.08–2.28 (1 H, m), 2.23 (3 H, s), 4.02–4.18 (4 H, m) and 6.53–6.90 (3 H, m); δ_C (50 MHz, $CDCl_3$): 13.6, 18.1, 18.7, 23.6, 30.5, 32.7 (d, $^{3}J_{PC}$ 5.5), 33.3, 43.4 (d, $^{2}J_{PC}$ 2.2), 52.8 (d, $^{3}J_{PC}$ 15.0), 56.3 (d, $^{1}J_{PC}$ 147.5), 66.5 (d, $^{2}J_{PC}$ 7.7), 66.6 (d, $^{2}J_{PC}$ 7.7), 120.5, 120.9, 124.2, 128.2, 136.1 (d, $^{3}J_{PC}$ 10.6) and 137.1; δ_P (161 MHz, $CDCl_3$) 29.94; *m/z* (ESI) 397.64 $(M + H)^+$, 419.65 $(M + Na)^+$.

*Diallyl-2,4,4,9-tetramethyl-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepin-2-ylphosphonate (**5bc + 5bc′**) (1 : 5 regio-isomeric mixture).* Yellow syrup; (Found: C, 62.57; H, 8.09; N, 7.74. Calc. for $C_{19}H_{29}N_2O_3P$: C, 62.62; H, 8.02; N, 7.69%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3355, 3019, 1599, 1520, 1466, 1384, 1319, 1215 and 1029; δ_H (200 MHz, $CDCl_3$) 1.28 (3 H, s), 1.32 (3 H, s), 1.63 (3 H, d, $^{3}J_{PH}$ 17.6), 1.75–1.85 (1 H, m), 2.12–2.19 (1 H, m), 2.23 (3 H, s), 4.55–4.66 (4 H, m), 5.15–5.44 (4 H, m), 5.86–6.03 (2 H, m) and 6.53–6.90 (3 H, m); δ_C (50 MHz, $CDCl_3$) 18.1, 23.6, 30.5, 33.3, 43.3, 52.8 (d, $^{3}J_{PC}$ 15.0), 56.4 (d, $^{1}J_{PC}$ 147.1), 67.1 (d, $^{2}J_{PC}$ 7.7), 67.2 (d, $^{2}J_{PC}$ 7.7), 118.0, 118.1, 120.6, 121.1. 124.3, 128.4, 132.9 (d, $^{3}J_{PC}$ 5.9), 133.0 (d, $^{3}J_{PC}$ 5.9), 135.9 (d, $^{3}J_{PC}$ 10.6) and 137.3; δ_P (161 MHz, $CDCl_3$) 30.95; *m/z* (ESI) 365.62 $(M + H)^+$, 387.62 $(M + Na)^+$.

*Diethyl-7,8-dichloro-2,4,4-trimethyl-2,3,4,5-tetrahydro-1*H*-benzo [*b*][1,4]diazepin-2-ylphosphonate (**5ca**).* Pale yellow syrup; (Found: C, 48.77; H, 6.44; N, 7.16. Calc. for $C_{16}H_{25}Cl_2N_2O_3P$: C, 48.62; H, 6.38; N, 7.09%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3421, 3019, 1647, 1542, 1489, 1215 and 1048; δ_H (400 MHz, $CDCl_3$) 1.32–1.37 (12 H, m), 1.59 (3 H, d, $^{3}J_{PH}$ 16.8), 1.77–1.82 (1 H, m), 2.17–2.24 (1 H, m), 4.13–4.25 (4 H, m), 6.72 (1 H, s) and 6.79 (1 H, s); δ_C (100 MHz, $CDCl_3$): 16.5 (d, $^{3}J_{PC}$ 6.6), 16.6 (d, $^{3}J_{PC}$ 5.9), 23.8, 30.8, 33.2, 43.3, 53.5 (d, $^{3}J_{PC}$ 13.9), 56.2 (d, $^{1}J_{PC}$ 148.2), 62.7 (d, $^{2}J_{PC}$ 8.1), 63.2 (d, $^{2}J_{PC}$ 7.3), 121.7, 122.1, 123.7, 123.9, 136.4 (d, $^{3}J_{PC}$ 11.0) and 137.1; δ_P (161 MHz, $CDCl_3$): 29.1; *m/z* (ESI): 395.12 $(M + H)^+$, 417.10 $(M + Na)^+$.

*Dibutyl-7,8-dichloro-2,4,4-trimethyl-2,3,4,5-tetrahydro-1*H*-benzo [*b*][1,4]diazepin-2-ylphosphonate (**5cb**).* Yellow syrup; (Found: C, 53.25; H, 7.43; N, 6.24. Calc. for $C_{20}H_{33}Cl_2N_2O_3P$: C, 53.22; H, 7.37; N, 6.21%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3421, 3019, 2964, 1607, 1488, 1385, 1215 and 1028; δ_H (500 MHz, $CDCl_3$): 0.93 (6 H, 2t, *J* 7.3), 1.29 (3 H, s), 1.33–1.42 (4 H, m), 1.37 (3 H, s), 1.58 (3 H, d, $^{3}J_{PH}$ 16.8), 1.62–1.67 (4 H, m), 1.75–1.79 (1 H, m), 2.16–2.21 (1 H, m), 4.05–4.12 (4 H, m), 6.69 (1 H, s) and 6.76 (1 H, s); δ_C (125 MHz, $CDCl_3$): 13.6, 18.8, 24.1, 30.9, 32.6 (d, $^{3}J_{PC}$ 5.5), 32.7 (d, $^{3}J_{PC}$ 5.5), 33.2, 43.2, 53.5 (d, $^{3}J_{PC}$ 13.6), 56.5 (d, $^{1}J_{PC}$ 148.1), 66.5 (d, $^{2}J_{PC}$ 8.2), 66.8 (d, $^{3}J_{PC}$ 7.3), 121.5, 122.1, 123.6, 123.9, 136.3 (d, $^{3}J_{PC}$ 9.9) and 137.0; δ_P (202 MHz, $CDCl_3$) 28.91; *m/z* (ESI) 383.32 $(M + H)^+$, 405.31 $(M + Na)^+$.

*Diallyl-7,8-dichloro-2,4,4-trimethyl-2,3,4,5-tetrahydro-1*H*-benzo [*b*][1,4]diazepin-2-ylphosphonate (**5cc**).* Yellow syrup; (Found: C, 51.64; H, 6.15; N, 6.74. Calc. for $C_{18}H_{25}Cl_2N_2O_3P$: C, 51.56; H, 6.01; N, 6.68%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3421, 3019, 1611, 1423, 1215 and 1019; δ_H (400 MHz, $CDCl_3$) 1.29 (3 H, s), 1.36 (3 H, s), 1.61 (3 H, d, $^{3}J_{PH}$ 17.2), 1.74–1.84 (1 H, m), 2.16–2.35 (1 H, m), 4.53–4.62 (4 H, m), 5.23–5.38 (4 H, m), 5.83–6.02 (2 H, m), 6.69 (1 H, s) and 6.77 (1 H, s); δ_C (50 MHz, $CDCl_3$) 24.1, 30.9, 33.2, 43.2 (d, $^{2}J_{PC}$ 1.1), 53.5 (d, $^{3}J_{PC}$ 13.9), 56.5 (d, $^{1}J_{PC}$ 147.1), 67.1 (d, $^{2}J_{PC}$ 7.7), 67.4 (d, $^{2}J_{PC}$ 7.0), 118.3, 118.4, 121.6, 122.2, 123.5, 124.1, 132.8, 132.9, 135.6 (d, $^{2}J_{PC}$ 10.6) and 137.1; δ_P (161 MHz, $CDCl_3$) 29.68; *m/z* (ESI) 420.31 $(M + H)^+$.

*Dimethyl-7,8-dichloro-2,4,4-trimethyl-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepin-2-ylphosphonate (**5cd**).* Yellow syrup; (Found: C, 45.83; H, 5.82, N, 7.66. Calc. for $C_{14}H_{21}Cl_2N_2O_3P$: C, 45.79; H, 5.76; N, 7.63%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3421, 3019, 2964, 1622, 1542, 1488, 1385, 1216 and 1031; δ_H (200 MHz, $CDCl_3$) 1.30 (3 H, s), 1.34 (3 H, s), 1.58 (3 H, d, $^{3}J_{PH}$ 17.2), 1.73–1.83 (1 H, m), 2.05–2.25 (1 H, m), 3.79 (3 H, d, $^{3}J_{PH}$ 10.4), 3.82 (3 H, d, $^{3}J_{PH}$ 10.3), 6.72 (1 H, s) and 6.81 (1 H, s); δ_C (50 MHz, $CDCl_3$) 23.9, 30.7, 33.2, 43.4, 53.4(d, $^{2}J_{PC}$ 7.7), 53.5 (d, $^{3}J_{PC}$ 13.9), 54.0 (d, $^{2}J_{PC}$ 7.0), 56.1 (d, $^{1}J_{PC}$ 148.6), 121.8, 122.2, 123.7, 124.1, 136.2 (d, $^{3}J_{PC}$ 10.9) and 137.1; δ_P (202 MHz, $CDCl_3$) 31.33; *m/z* (ESI) 367.17 $(M + H)^+$, 389.16 $(M + Na)^+$.

*Diethyl-2,4,4-trimethyl-8-nitro-2,3,4,5-tetrahydro-1*H*-benzo-[*b*][1,4]diazepin-2-ylphosphonate (**5da + 5da′**) (1 : 8 regio-isomeric mixture).* Pale yellow semi-solid; (Found: 51.81; H, 7.17; N, 11.23. Calc. for $C_{16}H_{26}N_3O_5P$: C, 51.75; H, 7.06; N, 11.31%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3421, 3367, 3019, 1614, 1519, 1216 and 1054; δ_H (200 MHz, $CDCl_3$) 1.26 (6 H, t, *J* 7.0), 1.39 (3 H, s), 1.59 (3 H, d, $^{3}J_{PH}$ 16.7), 1.57 (3 H, s), 1.76–1.85 (1 H, m), 2.37–2.53 (1 H, m), 4.01–4.21 (4 H, m), 6.43 (1 H, d, *J* 8.7) and 7.58–7.90 (2 H, m); δ_C (50 MHz, $CDCl_3$) 16.4 (d, $^{3}J_{PC}$ 5.5), 16.5 (d, $^{3}J_{PC}$ 5.5), 25.0, 32.0, 33.5, 43.1, 54.3 (d, $^{1}J_{PC}$ 11.7), 55.2 (d, $^{1}J_{PC}$ 145.6), 62.5 (d, $^{2}J_{PC}$ 8.0), 62.7 (d, $^{2}J_{PC}$ 7.3), 116.5, 117.4, 119.3, 132.3 (d, $^{3}J_{PC}$ 7.0),

138.9 and 145.3; δ_P (161 MHz, $CDCl_3$) 28.35; *m/z* (ESI) 372.67 $(M + H)^+$, 394.73 $(M + Na)^+$.

*Dibutyl2,4,4-trimethyl-8-nitro-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepin-2-ylphosphonate (**5db′**).* Pale yellow semi-solid; (Found: C, 56.10; H, 8.14; N, 9.96 Calc. for $C_{20}H_{34}N_3O_5P$: C, 56.19; H, 8.02; N, 9.83%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3420, 3367, 3019, 2964, 1593, 1518, 1319, 1216 and 1024; δ_H (400 MHz, $CDCl_3$) 0.87–0.95 (6 H, m), 1.27–1.61 (8 H, m), 1.38 (3 H, s), 1.57 (3 H, s), 1.60 (3 H, d, $^3J_{PH}$ 16.5), 1.78–1.83 (1 H, m), 2.40–2.48 (1 H, m), 3.74 (1 H, bs), 3.94–4.11 (4H, m), 4.18 (1 H, bs), 6.41 (1 H, d, *J* 8.8), 7.58 (1 H, d, *J* 2.5) and 7.67 (1 H, dd, *J* 8.7, 2.4); δ_C (100 MHz, $CDCl_3$) 13.6, 18.7, 18.8, 25.1 (d, $^4J_{PC}$ 1.10), 32.1, 32.6 (d, $^3J_{PC}$ 3.3), 32.7 (d, $^3J_{PC}$ 3.3), 33.6, 43.0, 54.3 (d, $^3J_{PC}$ 11.7), 56.9 (d, $^1J_{PC}$ 145.3), 66.3 (d, $^2J_{PC}$ 7.7), 66.5 (d, $^2J_{PC}$ 7.7), 116.5, 117.5, 119.3, 132.4 (d, $^3J_{PC}$ 6.6), 139.0 and 145.1; δ_P (161 MHz, $CDCl_3$) 25.7; *m/z* (ESI): 428.2 $(M + H)^+$, 450.2 $(M + Na)^+$.

*Diallyl-2,4,4-trimethyl-8-nitro-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepin-2-ylphosphonate (**5dc′**).* Pale yellow semi-solid; (Found: C, 54.75, 6.53. N, 10.59 Calc. for $C_{18}H_{26}N_3O_5P$: C, 54.68; H, 6.63; N, 10.63%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3421, 3367, 3020, 1593, 1519, 1484, 1320, 1215 and 1030; δ_H (500 MHz, $CDCl_3$) 1.38 (3 H, s), 1.59 (3 H, s), 1.62 (3 H, d, $^3J_{PH}$ 16.6), 1.80–1.84 (1 H, m), 2.47–2.53 (1 H, m), 4.38–4.58 (4 H, m), 5.20–5.30 (4 H, m), 5.80–5.88 (2 H, m), 6.40 (1 H, d, *J* 8.7), 7.58 (1 H, d, *J* 2.5) and 7.66 (1 H, dd, *J* 8.7, 2.5); δ_C (125 MHz, $CDCl_3$): 25.2, 32.0, 33.6, 43.0, 54.2, 57.0 (d, $^1J_{PC}$ 143.5), 66.8 (d, $^2J_{PC}$ 7.3), 67.0 (d, $^2J_{PC}$ 7.3), 116.4, 117.5, 118.2, 118.4, 119.4, 132.1 (d, $^3J_{PC}$ 5.9), 132.7 (d, $^3J_{PC}$ 5.3), 132.8 (d, $^3J_{PC}$ 5.3), 139.1 and 145.3; δ_P (202 MHz, $CDCl_3$) 29.1; *m/z* (ESI) 396.48 $(M + H)^+$, 418.43 $(M + Na)^+$.

*Dimethyl-2,4,4-trimethyl-8-nitro-2,3,4,5-tetrahydro-1*H*-benzo-[*b*][1,4]diazepin-2-ylphosphonate (**5dd + 5dd′**) (1 : 1 regio-isomeric mixture).* Yellow syrup; (Found: C, 49.87; H, 6.52; N, 12.22. Calc. for $C_{14}H_{22}N_3O_5P$: C, 49.98; H, 6.46; N, 12.24%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3420, 3360, 3019, 2923, 1592, 1518, 1318, 1215 and 1046; δ_H (400 MHz, $CDCl_3$) 1.28 (3 H, s), 1.38 (3 H, s), 1.70 (3 H, d, $^3J_{PH}$ 16.6), 1.79–1.87 (1 H, m), 2.15–2.45 (1 H, m), 3.68 (3 H, d, $^3J_{PH}$ 10.3), 3.79 (3 H, d, $^3J_{PH}$ 10.3), 6.42 (1 H, d, *J* 8.8) and 7.53–7.64 (2 H, m); δ_C (100 MHz, $CDCl_3$): 25.1, 31.2, 32.4, 43.2, 53.6 (d, $^2J_{PC}$ 8.1), 54.1 (d, $^2J_{PC}$ 7.3), 53.7 (d, $^3J_{PC}$ 11.0), 57.0 (d, $^1J_{PC}$ 149.7), 116.4, 118.2, 119.4, 135.1, 140.9 and 145.2; δ_P (161 MHz, $CDCl_3$): 30.80; *m/z* (ESI) 344.28 $(M + H)^+$, 366.25 $(M + Na)^+$.

*Diethyl-7-benzoyl-2,4,4-trimethyl-2,3,4,5-tetrahydro-1*H*-benzo-[*b*][1,4]diazepin-2-ylphosphonate (**5ea + 5ea′**) (3 : 4 regio-isomeric mixture).* Yellow syrup; (Found: C, 64.27; H, 7.30; N, 6.50. Calc. for $C_{23}H_{31}N_2O_4P$: C, 64.17; H, 7.26; N, 6.51%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3420, 3368, 3019, 1730, 1620, 1515, 1446, 1319 and 1215; δ_H (200 MHz, $CDCl_3$) 1.24–1.48 (12 H, m), 1.58 (3 H, d, $^3J_{PH}$ 16.9), 1.77–1.89 (1 H, m), 2.14–2.46 (1 H, m), 4.05–4.24 (4 H, m), 6.52 (1 H, d, *J* 8.7), 7.21–7.28 (2 H, m), 7.40–7.57 (3 H, m) and 7.69–7.74 (2 H, m); δ_C (50 MHz, $CDCl_3$): 16.4 (d, $^3J_{PC}$ 4.4), 16.6 (d, $^3J_{PC}$ 4.4), 24.6, 31.9, 33.5, 43.5, 54.0 (d, $^3J_{PC}$ 13.5), 56.5 (d, $^1J_{PC}$ 146.7), 62.9 (d, $^2J_{PC}$ 7.0), 63.4 (d, $^2J_{PC}$ 7.0), 117.9, 123.9, 126.1, 128.1, 130.0, 129.5, 131.4, 135.5, 138.9, 143.0 and 195.2; δ_P (161 MHz, $CDCl_3$) 29.08; *m/z* (ESI) 431.76 $(M + H)^+$, 453.77 $(M + Na)^+$.

*Dibutyl-7-benzoyl-2,4,4-trimethyl-2,3,4,5-tetrahydro-1*H*-benzo-[*b*][1,4]diazepin-2-ylphosphonate (**5eb + 5eb′**) (1 : 3 regio-isomeric mixture).* Yellow syrup; (Found: C, 66.70; H, 8.13; N, 5.68. Calc. for $C_{27}H_{39}N_2O_4P$: C, 66.65; H, 8.08; N, 5.76%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3421, 3392, 3018, 1641, 1593, 1480, 1320 and 1216; δ_H (200 MHz, $CDCl_3$) 0.94 (6 H, t, *J* 7.2), 1.19–1.75 (17 H, m), 1.82–2.41 (2 H, m), 4.00–4.15 (4 H, m), 6.52 (1 H, d, *J* 8.6), 7.23–7.30 (2 H, m), 7.40–7.56 (3 H, m) and 7.69–7.74 (2 H, m); δ_C (100 MHz, $CDCl_3$): 13.6, 18.7, 24.7, 31.2, 33.4, 32.6 (d, $^3J_{PC}$ 5.8), 32.6 (d, $^3J_{PC}$ 5.5), 43.5, 53.9 (d, $^3J_{PC}$ 13.5), 56.8 (d, $^1J_{PC}$ 146.4), 66.3 (d, $^2J_{PC}$ 8.0), 66.6 (d, $^2J_{PC}$ 7.7), 119.2, 123.9, 126.1, 128.1, 128.5, 129.5, 131.3, 135.4, 138.9, 143.0 and 195.5; δ_P (161 MHz, $CDCl_3$) 28.96; *m/z* (ESI) 487.94 $(M + H)^+$, 509.95 $(M + Na)^+$.

*Diallyl-7-benzoyl-2,4,4-trimethyl-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepin-2-ylphosphonate (**5ec + 5ec′**) (1 : 2 regio-isomeric mixture).* Yellow syrup; (Found: C, 66.13; H, 6.84; N, 6.21. Calc. for $C_{25}H_{31}N_2O_4P$: C, 66.07; H, 6.87; N, 6.16%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3420, 3370, 3019, 1641, 1521, 1476, 1422 and 1215; δ_H (200 MHz, $CDCl_3$) 1.38 (3 H, s), 1.50 (3 H, s), 1.61 (3 H, d, $^3J_{PH}$ 17.1), 1.79–1.88 (1 H, m), 2.35–2.51 (1 H, m), 4.43–4.63 (4 H, m), 5.14–5.49 (4 H, m), 5.78–5.97 (2 H, m), 6.51 (1 H, d, *J* 8.6), 7.23–7.29 (1 H, m), 7.39–7.54 (4 H, m) and 7.67–7.78 (2 H, m); δ_C (50 MHz, $CDCl_3$) 24.8, 31.9, 33.5, 43.4, 54.0 (d, $^3J_{PC}$ 12.4), 56.9 (d, $^1J_{PC}$ 144.9), 66.9 (d, $^2J_{PC}$ 8.0), 67.2 (d, $^2J_{PC}$ 7.0), 117.8, 118,1, 118.2, 124.1, 128.2, 128.1, 128.7, 129.5, 131.4, 132.9, 133.2, 138.9, 142.9 and 195.1; δ_P (161 MHz, $CDCl_3$): 29.79; *m/z* (ESI) 455.44 $(M + H)^+$, 477.29 $(M + Na)^+$.

*Dimethyl-7-benzoyl-2,4,4-trimethyl-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepin-2-ylphosphonate (**5ed + 5ed′**) (1 : 1 regio-isomeric mixture).* Yellow syrup; (Found: C, 62.75; H, 6.72; N, 6.91. Calc. for $C_{21}H_{27}N_2O_4P$: C, 62.68; H, 6.76; N, 6.96%); $\nu_{max}(CHCl_3)/cm^{-1}$ 3420, 3018, 2956, 1653, 1641, 1593, 1508, 1338, 1217, 1132 and 1053; δ_H (400 MHz, $CDCl_3$) 1.37 (3 H, s), 1.39 (3 H, s), 1.70 (3 H, d, $^3J_{PC}$ 16.6), 1.78–1.88(1 H, m), 2.20–2.40 (1 H, m), 3.74–3.85 (6 H, m), 6.69 (1 H, d, *J* 8.6), 7.23–7.27 (2 H, m), 7.43–7.54 (3 H, m) and 7.72–7.82 (2 H, m); δ_C (100 MHz, $CDCl_3$): 24.6, 30.9, 32.7, 43.1, 53.2 (d, $^2J_{PC}$ 7.3), 53.4, 56.9 (d, $^1J_{PC}$ 149.6), 118.2, 123.9, 125.8, 128.1, 129.6, 130.3, 131.6, 133.4, 138.7, 141.9 and 195.2; δ_P (161 MHz, $CDCl_3$): 31.5; *m/z* (ESI): 403.62 $(M + H)^+$, 425.69 $(M + Na)^+$.

Bioassay of synthesized BDPs. The inhibitory activity of cysteine protease inhibitor (CPI) against clostripain was assayed spectrophotometrically.[18] Clostripain was activated in 10 mM Tris HCL buffer, pH 7.4, containing 1 mM $CaCl_2$ and 2.5 mM DTT for 3 h at 37 °C. After activation, clostripain (25 nM) was added to enzyme buffer (100 mM Tris HCl buffer, pH 7.4) containing the substrate BAPNA (500 μM) in the presence and absence of CPI. Formation of product (*p*-nitroaniline) was monitored by the increase in absorbance at 410 nm.

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