

Conjugate Additions of *E*-alkenylphosphonates to Lithiated Schöllkopf's bislactim ether: Stereocontrolled Access to *anti* 2-Amino-3-substituted-4-phosphonobutanoic Acids

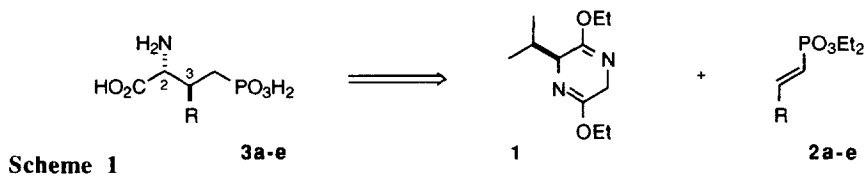
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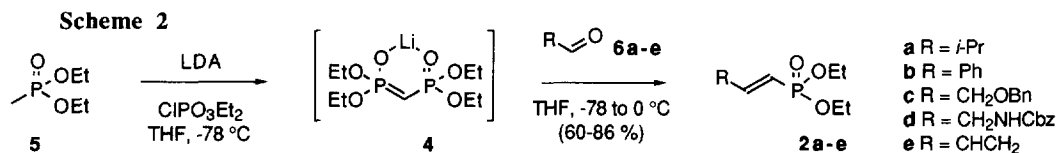
Abstract: Highly face-selective Michael addition of lithiated Schöllkopf's bislactim ether (derived from *cyclo*-[L-val-gly], **7**) to *E*-alkenylphosphonates **2a-d** and 1,3-butadienylphosphonate **2e** allows a direct and stereocontrolled access to the excitatory amino acid analogues 2,3-*anti*-2-amino-3-substituted-4-phosphonobutanoic acids **14a-d** and 2-amino-6-phosphono-4-hexenoic acid **15**. The relative stereochemistry was assigned from a NMR study of cyclic derivatives **16**, **17** and **19**.
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Glutamic acid is the major excitatory neurotransmitter in the Central Nervous System, and is considered to be involved in events leading to neuronal plasticity and degeneration.¹ Thus, the potential therapeutic use of compounds capable of modulating glutamate receptor function has been recognised for more than a decade. Excitatory amino acid (EAA) receptors have been classified into two major types: the ionotropic (iGluRs) and metabotropic receptors (mGluRs).² EAA bind to at least four subtypes of iGluRs named according to the known exogenous agonists *N*-methyl-D-aspartate (NMDA), quisqualic acid (Q/AMPA), kainic acid (K), and 2-amino-4-phosphonobutanoic acid (AP4). While there are many structure-activity relationship studies on NMDA, Q/AMPA and K receptors, less attention has been paid to the AP4 and mGluRs. Thus, screening and chemical optimisation of lead compounds are still required as the source of new selective agonists and antagonists that may serve as pharmacological tools to support further research in this field.³ As part of a project directed to the design of new bioactive phosphonates⁴ we report now the diastereoselective synthesis of 2,3-*anti* 2-amino-3-substituted-4-phosphonobutanoic acids, prepared in order to study the stereochemical requirements for a more selective binding to mGluRs and AP4 receptors.

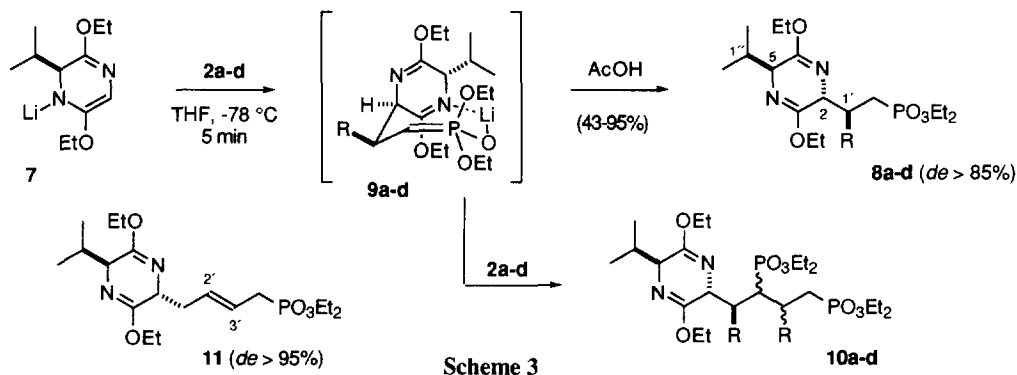
Although conjugate additions to α,β -unsaturated carbonyl compounds constitute a powerful method in asymmetric synthesis⁵ and α,β -unsaturated phosphoryl compounds act as Michael acceptors with a variety of nucleophiles,⁶ diastereoselective 1,4-additions to alkenylphosphonates have received little attention.^{6,7} We have recently reported the face-selective 1,4-addition of lithiated Schöllkopf's bislactim ether **1** to *Z*- and *E*-prop-2-enylphosphonates, as a direct approach to enantiopure 2-amino-3-methyl-4-phosphonobutanoic acids.⁸ The high stereoselectivity found in this process prompted us to explore the extension of this reactivity to other alkenylphosphonates **2a-e**, carrying different functional groups on the prochiral center (R = alkyl, alkenyl, aryl, alkoxy or amino) that could enable a stereocontrolled access to agonists of mGluRs and AP4 receptors, the 2-amino-3-substituted-4-phosphonobutanoic acids **3a-e** (see scheme 1).



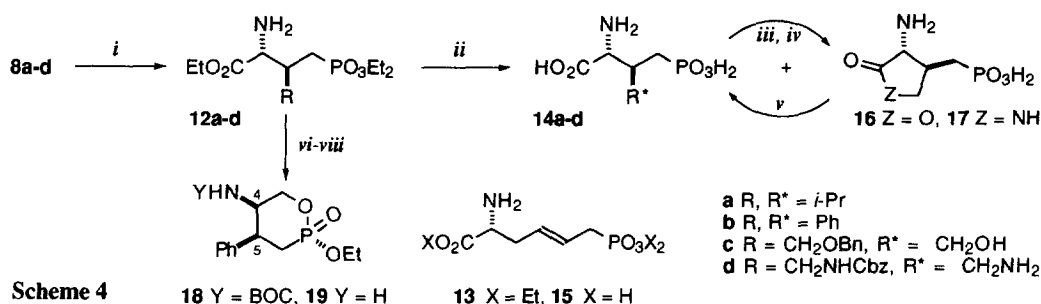
To this end, the required α,β -unsaturated phosphoryl compounds were prepared by a modified Wadsworth-Horner-Emmons olefination.⁹ Condensation of the anion derived from the methylenbisphosphonate **4** (generated *in situ* by treatment of the lithium salt of methylenbisphosphonate **5** with diethylchlorophosphate) ¹⁰ with several representative aldehydes (**6a-e**)¹¹ afforded the *E*-1-alkenylphosphonates **2a-e** in good yields.¹²



Slow addition of alkenylphosphonates **2a-d** over a solution of the lithium salt **7** ¹³ at -78°C led to mixtures of Michael adducts (**8a-d** plus other diastereoisomers, see scheme 3) in moderate to good yields. Phosphonates with alkyl substituents (**2a,c,d**) were found to be better acceptors, but gave rise to Michael adducts with lower yields (43-64%) than that one carrying a phenyl group (**2b**, 89%). The low chemical yields are due to a further capture of the initially formed adduct anions **9a-d** by second molecules of the alkenylphosphonates, originating mixtures of diastereoisomeric 1:2 addition products **10a-d**. The anionic dimerization was reduced simply by using two equivalents of **7**, thus improving the isolated yield of the 1:1 adducts to 61-95%.¹⁴ Integration of the pairs of doublets corresponding to the isopropyl groups in the ¹H NMR spectra of all the mixtures of adducts revealed a high asymmetric induction in the formation of the new chiral centers, as previously found for the additions to *E*-prop-2-enyl-phosphonates.^{4,8} Thus, the diastereomeric excesses (*de*) of the 2,5-*trans*-2,1'-*anti* adducts **8a-d** over the rest of diastereoisomers were all greater than 85%.¹⁵ Butadienylphosphonate **2e** resulted in being the best acceptor of the series, reacting regioselectively with **7** affording 1,6-addition products **16** with high stereoselection. Thus, after flash chromatography, a mixture of 1,6-adducts was isolated in good yield (39 or 65%, by using 1 or 2 eq. of **7**, respectively), and according to the ¹H NMR spectrum, it contained the 2,5-*trans* adduct **11** with a 95% of *de* over its 2,5-*cis* epimer.¹⁷



The separation of diastereomeric mixtures could be achieved by medium pressure liquid chromatography (mixtures AcOEt-hexane, SiO₂ 230-400 mesh) to provide products of high purity (*de*>98%) on a multigram scale. Mild acid hydrolysis of the bislactim ether gave rise to the amino esters **12a-d** and **13** in very good yields after chromatography (AcOEt to remove the valine ester, followed by AcOEt/MeOH 10:1). Vigorous acid hydrolysis of the amino esters allowed, after purification by reverse phase chromatography (H₂O, RP-18 230-400 mesh), the isolation of the amino acids **14a,b** and **15** as their hydrochloride salts in excellent yields (see scheme 4). On the other hand, hydrolysis of amino ester **12c** took place with simultaneous cyclization to lactone **16**, while amino ester **12d** afforded a mixture 2:1 of the corresponding amino acid **14d** and lactam **17**. By heating this mixture in boiling NH₄OH the amino acid : lactam ratio decreased to 1:7. Amino acid **14c** was obtained as its potassium salt by basic hydrolysis of lactone **16**.¹⁸



Reagents and conditions:

i: HCl 0.25 M : THF 1:1, rt, 8-10 h (75-94%). *ii*: HCl 12 M, reflux, 6 h (89-92%). *iii*: NH₄OH 25M, reflux 48 h. *iv*: Dowex-H, NH₄OH 1% (94%). *v*: 4 eq KOH, H₂O, rt, 1h (88%). *vi*: (BOC)₂O, Na₂CO₃, NaHCO₃, dioxane:H₂O 1:1, rt, 3h (98%). *vii*: LiBH₄, THF, rt, 16h, (78%). *viii*: TFA:H₂O 1:1, rt, 5h (92%).

Compounds **16** and **17** showed in their ¹H NMR spectra a pattern of signals suitable for the study of their relative stereochemistry by NOE difference spectroscopy, thus enabling the determination of the stereochemical course for the Michael addition to functionalized alkenylphosphonates **2c,d**. For cases **a** and **b**, the relative stereochemistry was confirmed by conversion of **8b** to an amino-oxaphosphorinane derivative, as was previously reported for the additions to 2-prop-enylphosphonates.⁸ As expected, chemoselective reduction of the carboxylic ester of a *N*-Boc derivative of amino ester **12b** (LiBH₄ at rt) took place with simultaneous cyclization to the oxaphosphorinane **18**, which was isolated as one single diastereomer ([α]_D²⁰ = +84.3 [CH₂Cl₂, c = 1.5]) in good yield. Finally, cleaving of the carbamate group gave rise to compound **19**, which showed a single chair conformation in its ¹H NMR spectrum (500 MHz, D₂O, rt). After corroborating the ¹H NMR assignments by COSY experiments, on the basis of the sets of observed NOEs (supported by force field and semiempirical calculations,¹⁹ see figure 1), a *trans* configuration was concluded for compounds **16** and **17**, while a 4,5-*cis* stereochemistry was assigned for **19**. All these results allow us to assume an 2,1'-*anti* configuration for major adducts **8a-d**.

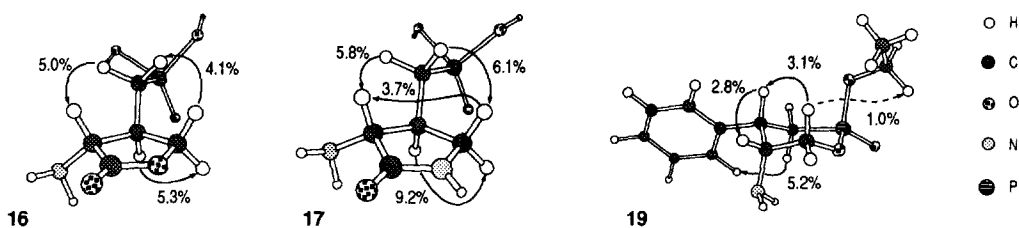


Fig. 1. PM3-optimised minimum energy conformations (MMX force field) found for models of compounds **16**, **17** and **19**, showing characteristic NOEs.¹⁹

Thus, the stereoselectivity of the conjugate addition of lithiated Schöllkopf's bislactim ether to *E*-alkenylphosphonates **2a-d** is similar to that previously encountered for prop-2-enylphosphonates,⁸ and also concordant with the stereochemical course reported for the corresponding additions to α,β-unsaturated esters and nitroolefins.²¹ A tight lithium chelation, giving rise to cyclic transition states, and the tetrahedral nature of phosphoryl group must account for the much higher facial diastereoselectivity observed in the additions to alkenylphosphonates.

In conclusion, Michael addition of a lithiated Schöllkopf's bislactim ether to *E*-alkenylphosphonates allows a direct and stereocontrolled access to a variety of *anti* 2-amino-3-substituted-4-phosphonobutanoic acids, potential agonist of AP4 and mGluRs, while addition to 1,3-butadienylphosphonate selectively leads to 2-amino-6-phosphono-4-hexenoic acid, that could act as a selective antagonist of the NMDA receptor. Evaluation of the biological activity of these compounds is currently under progress.

ACKNOWLEDGEMENTS

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- Tetraethyl methylenediphosphonate can also be purchased from Aldrich Chemical Co.
- Monobenylation of ethylene glycol followed by oxidation with PCC afforded aldehyde **6c**. Aldehyde **6d** was obtained by acidic hydrolysis (HCl 2M, 7h) of the benzyloxycarbonyl derivative aminoacetaldehyde dimethylacetal.
- All new compounds have been isolated in a pure analytical form after chromatography (on SiO₂ or RP-18), and their spectral data (EIMS or FABMS, NMR and IR) were consistent with the proposed structure. Alkenylphosphonates were obtained with >98% *E*-configuration (¹H NMR analyses). Spectral data obtained for compounds **2** are in full agreement with previously reported: **2a**: Babler, J.H.; Kiddle, J.J. *J. Org. Chem.* **1993**, *58*, 3572. **2d**: Xu, Y.; Jin, X.; Huang, G.; Huang, Y. *Synthesis* **1983**, 556.
- (3*S*)-2,5-dithoxy-3-isopropyl-3,6-dihydropyrazine ((+)-**1**) was prepared from glycine and L-valine, by treatment of *cyclo*[(2*S*)-val-gly] with triethyloxonium tetrafluoroborate. See Rose, J.E.; Leeson, P.D.; Gani, D. *J. Chem. Soc. Perkin Trans 1* **1995**, 157. Alternatively, both enantiomers of the related 2,5-dimethoxy-3-isopropyl-3,6-dihydropyrazine can be purchased from E. Merck.
- The excess of Schöllkopf's reagent could be recovered, and showed no racemization.
- In addition to **8a-d**, the mixtures of Michael adducts contained the 2,5-*trans*-2,1'-*syn* and 2,5-*cis*-2,1'-*anti* isomers. Accordingly with the ¹H NMR spectra of the mixtures, the typical ratio between 2,1'-*anti* : 2,1'-*syn* : 2,5-*cis* adducts were *ca* 50 : 3 : <1. Thus, adducts **8a-d** were formed with high asymmetric induction with respect to the two stereocenters (*de* > 98% at C3, and > 89% at C1'). Nevertheless, the stereoselectivity of the addition was found to be markedly dependent on the reaction temperature. As an example, when **2b** was added to **7** at 0 °C, although the mixture of adducts was isolated with similar yield, **8b** was obtained with a *de* of 58% over the 2,5-*trans*-2,1'-*syn* epimer, and a *de* of 77% over the 2,5-*cis*-2,1'-*anti* isomer.
- Other 1,6-additions of ketone or aldehyde enolates to butadienyl phosphonate **2e** have been reported. See Darling, S.D.; Muralidharan, F.N.; Muralidharan, V.B. *Tetrahedron Lett.* **1979**, *30*, 2757, and references cited therein. Michael addition of lithiated Schöllkopf's bislactim ether to 2,4-pentanodioates also proceed with high selectivity, see Pettig, D.; Schöllkopf, U. *Synthesis* **1988**, 173.
- The relative configuration of substituents at pyrazino ring can be deduced from the coupling constant between H2 and H5. Thus, for compounds with a 2,5-*trans* configuration (**8a-d** and **11**) H5 resonance appears as a quasi triplet (⁵J_{H2H5} ~3.5 Hz, typical for a *trans* relation) with δ between 3.50-3.91 ppm, while disatereoisomers with 2,5-*cis* configuration show this absorption as a double doublet (³J_{H5H1'} ~3.5 Hz but ⁵J_{H2H5} ~7.5 Hz, typical for a *cis* relation) at similar δ . Unfortunately, configuration at double bond of 1,6-adducts could not be confirmed, but a 2',3'-*trans* relation is assumed on the basis of the similitude with the pentanodioate's behaviour reported by Schöllkopf¹⁶ and unpublished results from this laboratory.
- Salient data for the amino phosphonic acids: the ¹³C NMR spectra (50 MHz, D₂O, rt) of compounds **14a-d**, **16** and **17** exhibit typical signals for C1 (171.1-183.2), C2 (54.9-59.7, d, ³J_{CP}~ 3.0-16.0 Hz), C-3 (36.5-42.4, d, ²J_{CP}~ 2.0-3.7 Hz), C4 (25.3-31.8, d, ¹J_{CP}~ 128.3-136.0 Hz). ¹³C NMR (50 MHz, D₂O, rt) data for **15**: 32.5 (C-6, d, ¹J_{CP}= 132.3 Hz); 33.9 (C-3); 53.4 (C-2, d, ⁵J_{CP}= 4.1 Hz); 127.7 (C-4, d, ³J_{CP}= 14.9 Hz); 128.0 (C-5, d, ²J_{CP}= 10.8 Hz); 172.5 (C-1). **14a**: [α]_D²⁰ = -10.5 (H₂O, c = 0.3). **14b**: [α]_D²⁰ = -2.0 (H₂O, c = 1.4). **14c**: [α]_D²⁰ = -5.8 (KOH 0.1M, c = 3.3). **15**: [α]_D²⁰ = +17.3 (HCl 2.0M, c = 1.9).
- Minimum energy conformations for compounds **16**, **17** and **19** were located using MMX force field as implemented in PCModel v5.^{20a} The geometries of the most important conformers were fully optimised by semiempirical molecular orbital calculations, using the MNDO, AM1 and PM3 Hamiltonians included in MOPAC 7.0.^{20b} The refined geometries in the gas phase were in agreement with the conformations in solution deduced from ¹H NOE spectroscopy. For the oxaphosphorinane **19**, rotamers must account for the observed NOEs (showed with dashed arrows in figure 1) between H3,5 and methylene protons of the ethoxy chain.
- a. PCModel version 5. Molecular Modelling Software, from Serena Software, Bloomington, IN 47402, USA. b. Stewart, J.J.P. MOPAC 7.0. QCPE program 455, 1994. All calculations were performed on a Power Macintosh 7500/100.
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