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Structure-Based Design and Synthesis of Phosphinate Isosteres of Phosphotyrosine for Incorporation in Grb2-SH2 Domain Inhibitors. Part 2

Clive V. Walker,[a,*] Giorgio Caravatti,[b] Alastair A. Denholm,[a] John Egerton,[a] Alex Faessler,[a] Pascal Furet,[b] Carlos García-Echeverría,[b] Brigitte Gay,[b] Ed Irving,[a] Ken Jones,[a] Amanda Lambert,[a] Neil J. Press[a] and John Woods[a]

[a]*Novartis Horsham Research Centre, Wimblehurst Road, Horsham, West Sussex, RH12 5AB, UK*
[b]*Novartis Pharmaceuticals, Inc., Therapeutic Area Oncology, CH-4002 Basel, Switzerland*

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Abstract—A series of novel phosphinates, derived from 4-phosphonomethylphenylalanine, are described as isosteres of phosphotyrosine. Benzyl (or alkyl) phosphinomethylphenylalanine derivatives were prepared by alkylation of an amino acid P-H phosphinate.

The signal transduction processes mediated by Grb2 (growth factor receptor bound protein 2) are needed for the activity of growth factors and/or oncogenes that are responsible for cell growth.[1] Deregulation of growth factor signalling leads to rapid cell growth and is central to many tumours.[2] Thus, control of this signal transduction process, by antagonising the protein–protein interactions of Grb2-SH2 with activated receptors, was pursued to identify novel anti-tumour agents.

Central to this mechanism is the binding of the Grb2-SH2 domain to a peptide sequence containing phosphotyrosine (pY).[3] The design of novel peptidomimetic sequences that are highly potent antagonists of this interaction in vitro has been described.[4] However, replacement of the central pY recognition element remains a highly desirable target and the design and synthesis of pY mimics with improved properties have been investigated increasingly over recent years.[5] The known amino acid phosphonomethyl Phe (Pmp) is a stable phosphotyrosine mimic[6] which still contains most of the key recognition elements responsible for activity.

However, this compound and its synthetically useful derivatives (for example **1**) still represent highly charged species. We were interested in phosphinate derivatives (Fig. 1) where one charge has been replaced by a neutral group. The rationale, molecular modelling and biological results are described in Part 1 of this work. In Part 2 we describe the synthesis of these novel phosphinate isosteres **2**–**6** suitable for incorporation into peptidomimetic sequences.

Initially, we chose our synthetic strategy based on having a protected amino acid P-H synthon which could be alkylated at a late stage with different electrophiles. Thus, alkylation (Scheme 1) of the hypophosphorus acid derivative **7**[7] with 4-iodobenzyl bromide gave **8**. Zinc mediated Jackson coupling[8,6b] with either Boc-iodoAla-OMe[9] or Z-iodoAla-OBn[10] gave the protected hypophosphorus amino acid synthons **9a**,**b** in good yields. The latent P-H functionality was revealed using TMSCl[7] to give **10a**,**b** suitable for reaction with different electrophiles. Although no special precautions were taken the P-H compounds were routinely used immediately after deprotection to avoid any possibility of oxidation to the phosphonates.

The reaction of **10a**,**b** with electrophiles (Scheme 2) proceeded readily via either the P(III) species[11] or by direct alkylation[7b] using NaH as base to give the phosphinates **11b**–**d**. The hydroxymethyl compound **11a** was prepared by heating with paraformaldehyde.[12] For **11b** a slightly higher yield (68% versus 54%) was obtained using the P(III) method. Reaction of **10b** with benzal-

*Corresponding author. Tel.: +44-1403-272827; fax: +44-1403-323837; e-mail: clive.walker@pharma.novartis.com

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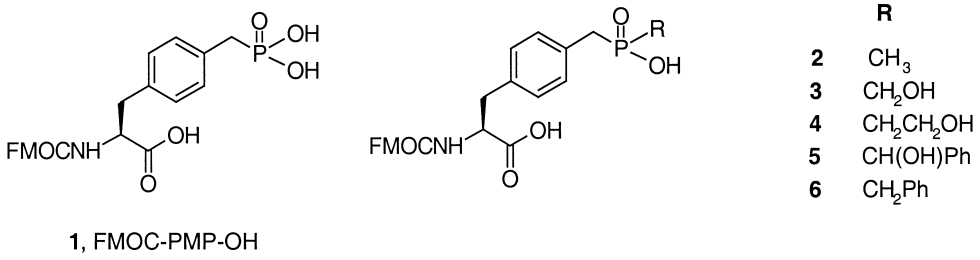
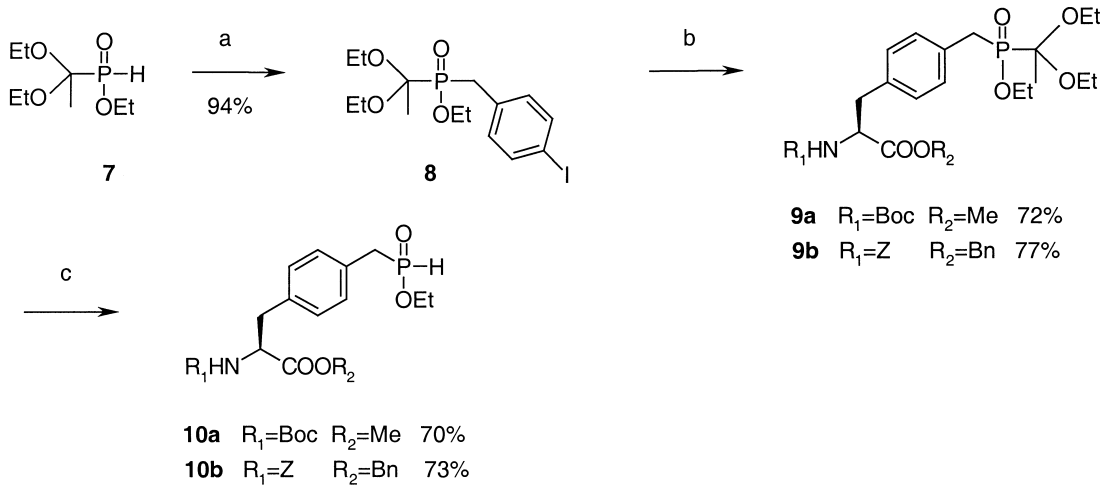
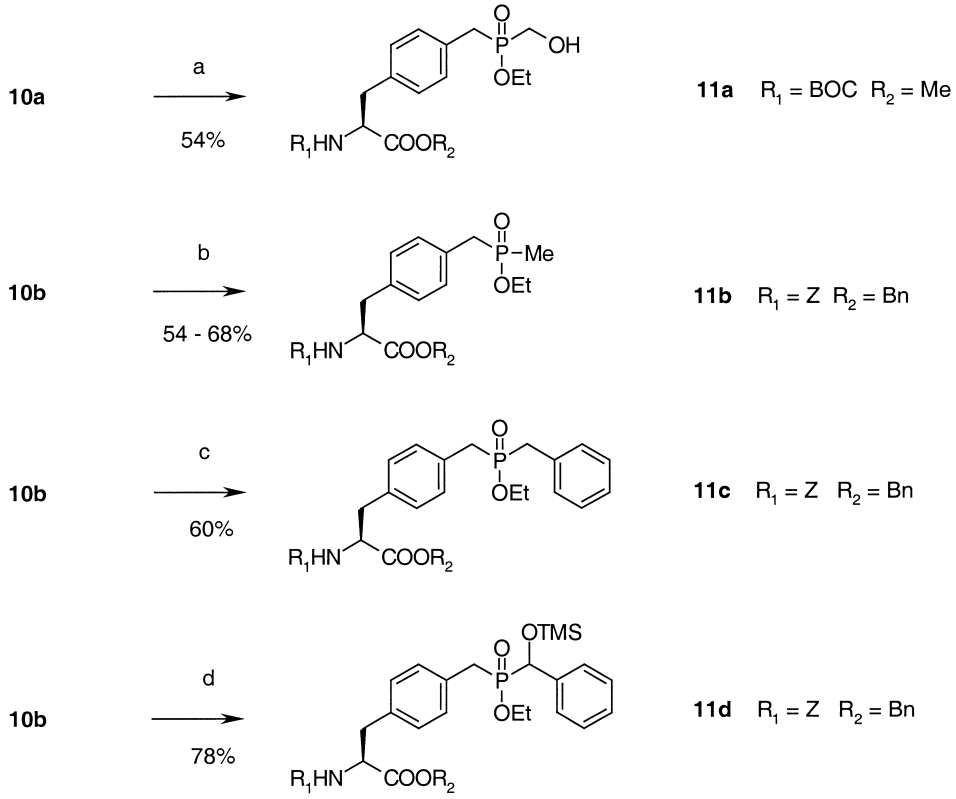


1, FMOC-PMP-OH

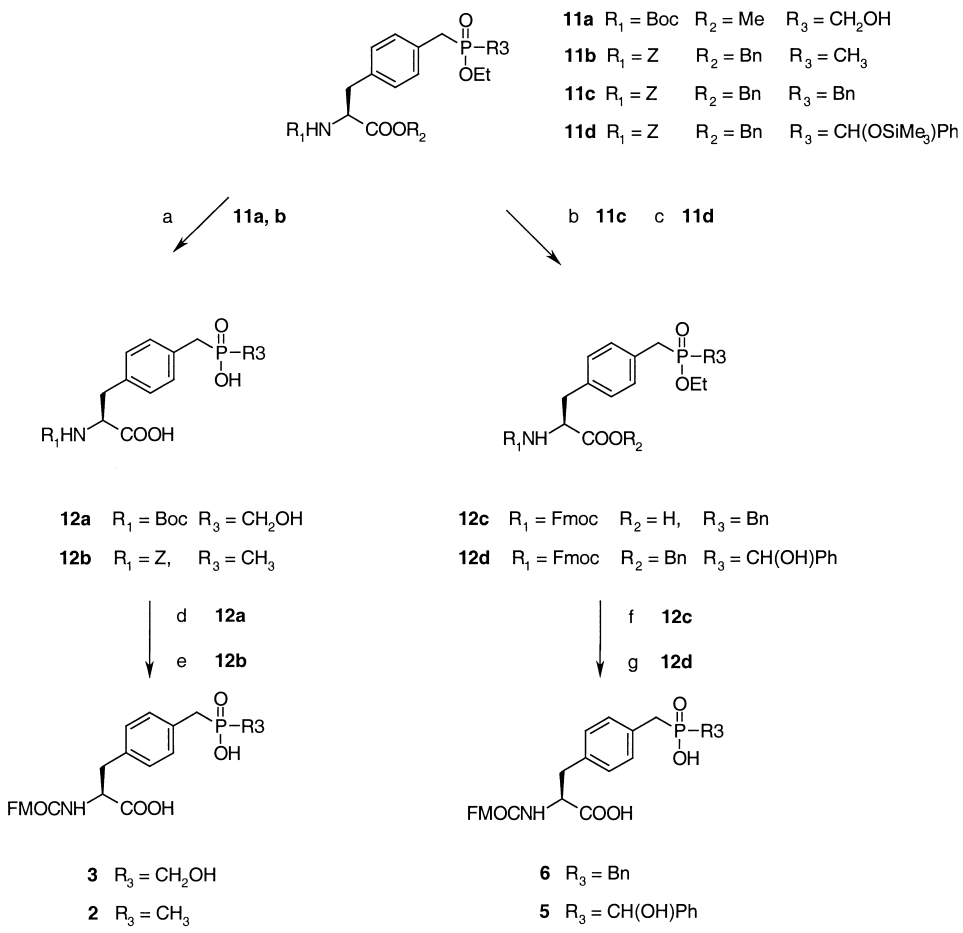
Figure 1.



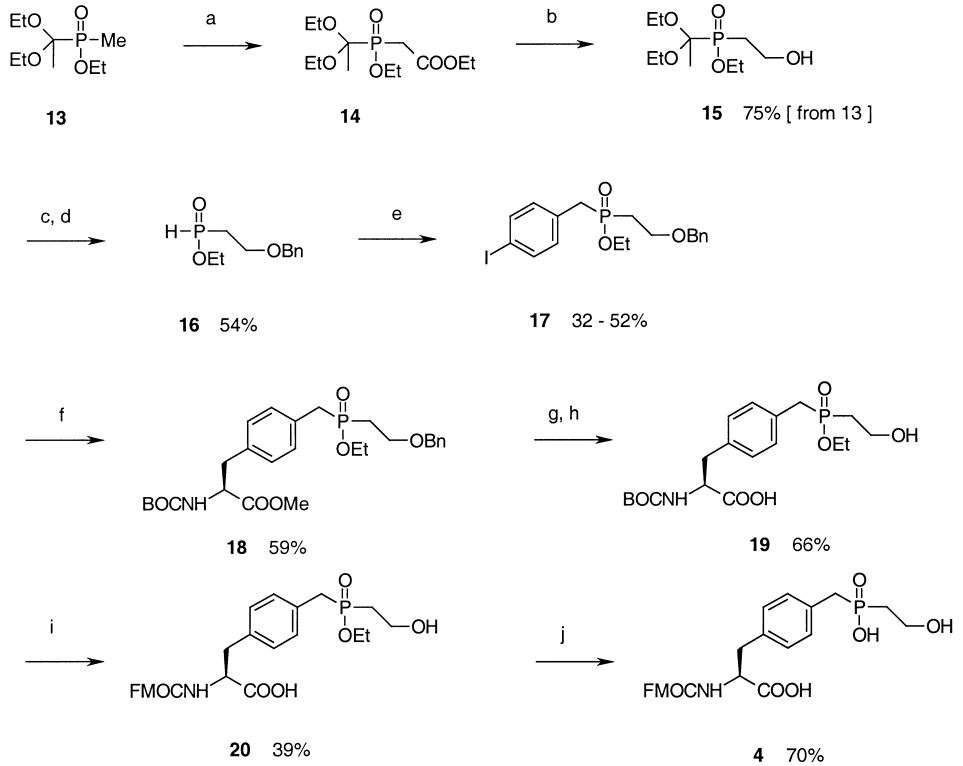
Scheme 1. (a) 4-Iodobenzylbromide, NaH, THF, −10 °C to rt; (b) (i) Boc-iodoAla-OMe, or Z-iodoAla-OBn, Zn, $BrCH_2CH_2Br$, TMSCl; (ii) (2-furyl)$_3$P, Pd_2dba_3, DMA–THF, 60 °C; (c) TMSCl, CH_2Cl_2 (or $CHCl_3$), EtOH, rt.



Scheme 2. (a) $(CH_2O)_n$, PhMe, Et_3N, 100 °C; (b) (i) NaH, MeI, THF, −10 °C to rt or (ii) TMSCl, *i*$PrEt_2N$, MeI, CH_2Cl_2; (c) TMSCl, *i*$PrEt_2N$, BnBr, CH_2Cl_2; (d) TMSCl, *i*$PrEt_2N$, PhCHO, CH_2Cl_2.



Scheme 3. (a) LiOH, EtOH–H_2O, rt; (b) (i) H_2, Pd–C; (ii) Fmoc-OSu, Na_2CO_3, dioxan; (c) (i) HBr–AcOH; (ii) Fmoc-OSu, Py, DMAP; (d) (i) TFA; (ii) Fmoc-Cl, Dioxan–H_2O; (e) (i) H_2, Pd–C; (ii) Fmoc-OSu, dioxan; (f) TMSI–CH_2Cl_2; (g) (i) 1,4-cyclohexadiene, Pd–C; (ii) TMSI–CH_2Cl_2.



Scheme 4. (a) LDA, $ClCO_2Et$, THF, −78 °C; (b) $LiBH_4$, Et_2O, 10–20 °C; (c) KHMDS, BnBr, THF, −78 °C to rt; (d) TMSCl, $CHCl_3$, EtOH, rt; (e) KHMDS, 4-I-BnBr, THF, −78 °C; (f) (i) Boc-iodoAla-OMe, Zn, $BrCH_2CH_2Br$, TMSCl; (ii) (2-furyl)$_3$P, Pd_2dba_3, DMA–THF, 60 °C; (g) H_2, Pd–C, EtOH; (h) LiOH, EtOH–H_2O; (i) (i) TFA; (ii) Fmoc-OSu, 10% aq Na_2CO_3, dioxan; (j) TMSI, CH_2Cl_2, rt.

dehyde gave the TMS ether **11d**, which was stable to chromatography, as a mixture of diastereomers.[12]

For solid-phase peptide synthesis we chose an Fmoc protection strategy. Thus, we deprotected both carboxyl and phosphinate esters of **11a**,**b** with LiOH to give **12a**,**b** and converted the Boc and Z groups to Fmoc to give **2** and **3** (Scheme 3). For **11c**,**d** we reversed this sequence and converted the N-terminus to Fmoc to give **12c**,**d** (the TMS ether of **11c** was cleaved under the acidic conditions used) followed by carboxyl and phosphinate deprotection, using TMSI mediated cleavage, to give **5** and **6**.

The Fmoc derivatives **2**, **3**, **5** and **6** were incorporated into Grb2-SH2 peptidomimetic sequences and the results are described in Part 1.

The previous route was not suitable for phosphinate **4** because, despite several attempts, alkylation of **10a**,**b** failed with β-hydroxy electrophiles. Thus, we used an alternative 'early alkylation' approach (Scheme 4) from the hypophosphorus acid synthon **13**.[7] Acylation and ester reduction gave the key hydroxyethyl phosphinate **15**. Protection of the alcohol as its benzyl ether and deprotection of the methyl ketal[7] revealed the latent P-H functionality in **16** which was alkylated with 4-iodobenzylbromide to give **17**.

The central Jackson coupling reaction with Boc-iodoAla-OMe[9] gave **18** and subsequent deprotection and functionalisation gave the target Fmoc phosphinic acid **4**. Although protection and deprotection reactions take up a significant part of this sequence it should be noted that other protection strategies, that were able to cope with the multi-functionality of **4**, were unsuccessful.

In conclusion, we have prepared the first examples of 4-(alkylphosphinomethyl)-phenylalanine derivatives, as phosphotyrosine isosteres, in Fmoc protected form suitable for solid phase synthesis of peptidomimetic sequences.

References and Notes

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3. The minimum recognition sequence, with micromolar affinity, for the Grb2-SH2 domain is pTyr-X_{+1}-Asn-NH_2 where asparagine at X_{+2} is critically important (Garcia-Echeverria, C. et al., Novartis Pharma Inc., Oncology, unpublished results).
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10. Z-Iodo-Ala-OBn: Literature preparation[13] of this amino acid is a two step procedure. We developed a one step procedure (amenable to scale-up) as follows: Z-Ser-OBn (8.2 g, 25 mmol) was placed in a flask under argon with triphenylphosphine (8.56 g, 2.6 mmol) and imidazole (2.36 g, 35 mmol). These were dissolved in acetonitrile (24.5 mL)/ether (40.9 mL) and cooled to 0 °C. Iodine (9.13 g, 36 mmol) was added slowly and the mixture stirred at 0 °C for 45 min, then diluted with ether (50 mL) and washed with $Na_2S_2O_3$ (aq) (20 mL), $CuSO_4$ (aq) (20 mL) and water (20 mL). The organic layer was dried ($MgSO_4$), concentrated and purified by flash chromatography eluting with 1:4 ethyl acetate:hexane. The title compound was obtained as white crystals, 7.15 g (64%), mp = 58–58.5 °C (lit.[13] 58 °C).
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12. Typical experimental procedures; **11a**: paraformaldehyde (1.10 g, 36.6 mmol) and triethylamine (0.51 mL, 3.7 mmol) were added to a solution of **10a** (2.82 g, 7.3 mmol) in toluene (15 mL) and the reaction heated at 100 °C for 2 h. The mixture was allowed to cool to room temperature, and concentrated in vacuo. The residue was pre-adsorbed onto silica gel and purified by flash chromatography, eluting with 4% MeOH/EtOAc to give **11a** as a colourless gum; ^{31}P NMR ($CDCl_3$) 49.2 ppm, TLC $R_F = 0.31$ (10% MeOH/ethyl acetate). **11b**: NaH (50 mg, 60% dispersion) was washed with hexane and added to a cooled (−10 °C) solution of **10b** (512 mg, 1.03 mmol) in THF (9 mL). The reaction mixture was treated with MeI (0.2 mL, 3 equiv) and stirred at −10 °C for 1 h and at rt for 0.8 h. The reaction was treated with EtOAc, washed with water, dried ($MgSO_4$) and evaporated. The crude material was purified by flash chromatography, eluting with EtOAc, to give **11b** as a colourless oil; ^{31}P NMR ($CDCl_3$) 51.17 ppm, TLC $R_F = 0.20$ (10% hexane/EtOAc). **11d**: A solution of **10b** (519 mg, 1.05 mmol) in CH_2Cl_2 (10 mL) was treated with TMSCl (3×0.25 mL) and iPr_2EtN (3×0.35 mL) over 2 h. After the final addition, benzaldehyde (0.24 mL) was added and the reaction stirred at rt for a further 2.5 h. The reaction was evaporated and the crude oil purified by flash chromatography, eluting with 25% EtOAc/CH_2Cl_2, to give **11d** as a colourless oil; ^{31}P NMR ($CDCl_3$) 49.7 and 45.9 ppm, TLC $R_F = 0.50$ (25% EtOAc/CH_2Cl_2).
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