Pergamon

Tetrahedron Letters 43 (2002) 4103–4106

TETRAHEDRON LETTERS

An expedient method for the solid-phase synthesis of α-aminoalkyl phosphonopeptides

Markéta Rinnová, Adel Nefzi and Richard A. Houghten*

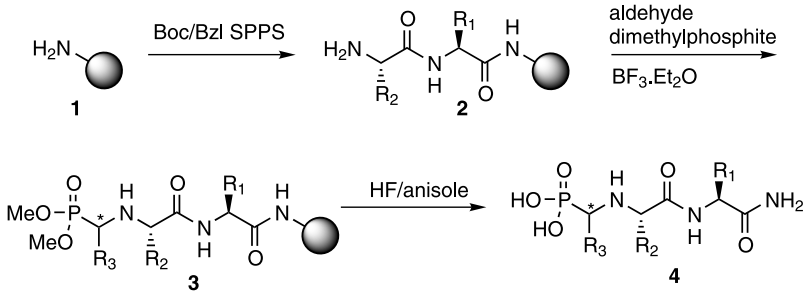
Torrey Pines Institute for Molecular Studies, 3550 General Atomics Court, San Diego, CA 92121, USA

Received 24 January 2002; accepted 22 March 2002

Abstract—The formation of α-amino phosphonate functionalities on the amino terminus of peptides utilizing solid-phase methodology is presented. The described method enables incorporation of diverse *N*-phosphonoalkyl and aryl moieties. © 2002 Published by Elsevier Science Ltd.

Miscellaneous α-amino phosphonate derivatives have been found to be highly potent inhibitors of a number of enzymes, e.g. protein–tyrosine phosphatases,[1] metalloproteases[2] or serine proteases.[3] Many of those compounds are based on peptides or peptidomimetic structures.[2,4] Amino phosphonopeptides are also known for their significant antibacterial activity.[5] Conventional methods for the preparation of α-amino phosphonates in solution are based on the three component reaction of an aldehyde, amine and dialkyl phosphite;[6] the presence of a Lewis acid as a catalyst significantly improves reactivity.[7] In general, these methods are non-stereospecific unless using a chiral-auxiliary[8] integrated into the synthetic scheme. Solid-phase synthetic approaches have been used for the convenient incorporation of various phosphonate[9] as well as phosphinate peptidomimetics[10] into peptide sequences and have been used in conjunction with combinatorial approaches[9a,10] to discover enzyme inhibitors. A solid-phase method has been presented in which a polymer bound H-phosphonate was reacted with imines in the presence of a Lewis acid to form an array of α-amino phosphonic acids.[11] The method we present here is based on an inverse concept and is aimed at the synthesis of a novel class of compounds by direct N-terminal modification of peptides by various aminoalkyl phosphonates.

Our primary goal was to optimize one-step, alkyl-phosphonation reaction conditions in conjunction with solid-phase methodologies to generate combinatorial libraries of alkyl phosphonopeptides. This approach involves the straightforward modification and adaptation of related solution chemistry procedures on the solid-phase. We examined the influence of various solvents (dichloromethane–DCM, *N*,*N*′-dimethylformamide–DMF, tetrahydrofuran–THF), optimized ratios of all reagents, and tested several Lewis acid catalysts: $LiClO_4$,[12] $BF_3{\cdot}Et_2O$,[2b] $Yb(OTf)_3$[13] and Me_3SiCl.[14] The general synthetic protocol used is



Scheme 1.

Keywords: phosphonopeptides; α-amino phosphonates; solid-phase synthesis.

* Corresponding author.

0040-4039/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0040-4039(02)00695-0

shown in Scheme 1. Thus, *p*-methylbenzhydrylamine (MBHA) resin-bound dipeptides **2** were prepared by parallel solid-phase peptide synthesis (SPPS).[15] Following drying of the resin, dry DCM (4 ml/100 mg resin) was added together with 4 Å sieves (0.5 g/4 ml DCM), an aldehyde (10 equiv.) and dimethyl phosphite (10 equiv.), and the resin was shaken for 2 min. Then $BF_3{\cdot}Et_2O$ (3 equiv.) was added and the mixture with the resin was shaken for 16 h at rt. Following decantation, the treatment was repeated. Before final cleavage of the dimethylester **3** using HF (0°C, 7 h), the resin was washed (DCM, DMF, DCM and MeOH) and dried. Following extraction and lyophilization, the products **4** were obtained as white solids. A side product **5**, formed by substitution of a hydroxy group of a phosphonate with fluorine, was detected following HF cleavage. This modification added a chiral center on phosphorus and thus increased the complexity of the diastereomeric mixture. Individual compounds were difficult to separate for exact determination of ratios of **4** to **5**. Estimated content of fluorinated products varied from 30 to 50%. However, the monofluoro-derivative is not stable in alkali solution and it is easily transformed into the desired phosphonate (1 mM aqueous solution of NaOH, 5 h). We applied this treatment, followed by neutralization (10% aqueous AcOH), desalting on Sephadex and lyophilization which provided the desired non-fluorinated products. We investigated the dealkylation of phosphonate diesters **3**. Treatment of this intermediate using HF for 1.5 h (0°C) provided a mixture of a free phosphonate **4** along with a monomethyl ester derivative. When the HF treatment was prolonged to 7 h we obtained exclusively dealkylated amino phosphonates **4**. Using Me_3SiBr in a modified two-step procedure,[16] known for dealkylation of phosphonoesters in solution,[17] followed by a 1.5 h treatment with HF, we obtained equivalent results. We tested our synthetic protocol on a range of dipeptides and aldehydes. The results are summarized in Table 1. The products were characterized by Electrospray LC–MS under ESI conditions, ^1H, ^{13}C and ^{31}P NMR (diastereomer configurations were not attributed).[18]

Using DMF instead of DCM favored concurrent cyclization and formation of a 4-imidazolidinone **6** (Fig. 1) from an imine-intermediate and a neighbor amide which we have described elsewhere.[19] The reactivity of aromatic aldehydes is in general lower than that of aliphatic aldehydes. Sterically hindered 2,5-dimethylbenzaldehyde provided approximately 5% of

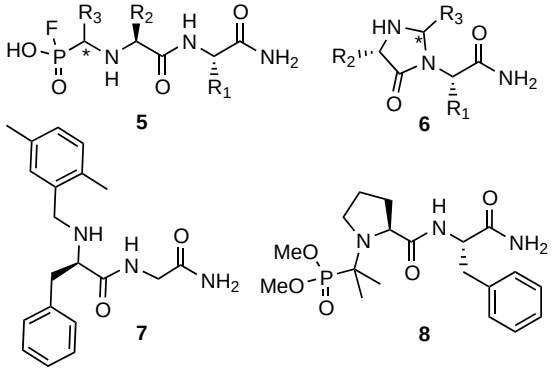
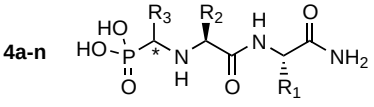


Figure 1.

Table 1.

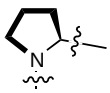


Entry	R_1	R_2	R_3	Yield[a] (%)	Ratio of diastereomers[b]	MW (expected/found)
4a	$PhCH_2$	$PhCH_2$	Ph	85	4:1	481.2/482.3
4b	$PhCH_2$	$HOOCCH_2$	Ph	79	2:3	449.2/450.4
4c	$PhCH_2$	$-(CH_2)_3-$[c]	Ph	82	9:1	431.2/432.4
4d	$PhCH_2$	CH_3	Ph	83	1:2	405.2/406.4
4e	$PhCH_2$	CH_3	Pr	88	1:4	371.2/372.3
4f	$PhCH_2$	$HOOCCH_2$	Pr	86	n.d.	415.2/416.3
4g	$PhCH_2$	$PhCH_2$	Pr	92	n.d.	447.2/448.1
4h	$PhCH_2$	$(CH_3)_2CH_2$	Bu	80	2:3	413.2/413.9
4i	CH_3	$PhCH_2$	Bu	71	n.d.	385.2/385.6
4j	CH_3	$PhCH_2$	Ph	53	n.d.	405.2/405.6
4k	CH_3	$PhCH_2$	Pr	65	n.d.	371.2/371.6
4l	H	$PhCH_2$	Bu	88	5:3	371.2/371.6
4m	$PhCH_2$	$-(CH_2)_3-$[c]	$iPrC_6H_4$	75	1:4	473.5/474.2
4n	$PhCH_2$	CH_3	$iPrC_6H_4$	70	4:5	447.2/448.4

[a] Yield of crude product based on resin substitution.

[b] Stereochemistry was not assigned (nd: not determined).

[c] $-(CH_2)_3-$:



the desired phosphonate with the major product identified by mass spectra as the product **7** resulting from reductive alkylation. The mechanism of this transformation is not completely clear, but reductive methylation of amines using formaldehyde in presence of phosphorous acid has been reported.[20] We also obtained significantly different results using various Lewis acids as phosphonation catalysts. We obtained very little or no phosphonate products using Me_3SiCl and $Yb(OTf)_3$. In both cases, these conditions provided the above-mentioned imidazolidinone side products. Finally, the best results were obtained using $BF_3{\cdot}Et_2O$. Reaction in the presence of $LiClO_4$ lead to the same results, but the reaction rate was much lower. Also, reactivity in DCM was greater than in THF, possibly due to the better swelling properties expected for the resin in DCM. When acetone was used in the presence of $LiClO_4$,[21] the dimethylester phosphonate **8** (Fig. 1) was obtained almost exclusively under the standard conditions.

In summary, we have optimized a solid-phase method for the generation of diverse α-amino alkyl or aryl phosphonates derived from peptides. The non-stereoselective method enables the preparation of libraries based on peptide or non-peptide phosphonates for use in the screening of potential enzyme inhibitors.

Acknowledgements

This work was funded by NCI 1P01CA 78040 (Houghten).

References

1. Burke, T. R., Jr.; Zhang, Z.-Y. *Biopolymers (Peptide Sci.)* **1998**, *47*, 225–241.
2. (a) Bird, J.; De Mello, R. C.; Harper, G. P.; Hunter, D. J.; Karran, E. H.; Markwell, R. E.; Milles-Williams, A. J.; Rahman, S. S.; Ward, R. W. *J. Med. Chem.* **1994**, *37*, 158–169; (b) De Lombaert, S.; Blanchard, L.; Stamford, L. B.; Tan, J.; Wallace, E. M.; Satoh, Y.; Fitt, J.; Hoyer, D.; Simonsbergen, D.; Moliterni, J.; Marcopoulos, N.; Savage, P.; Chou, M.; Trapani, A. J.; Jeng, A. Y. *J. Med. Chem.* **2000**, *43*, 488–504.
3. Jackson, D. S.; Frase, S. A.; Ni, L.-M.; Kam, Ch.-M.; Winkler, U.; Johnson, D. A.; Froelich, C. J.; Hudig, D.; Powers, J. C. *J. Med. Chem.* **1998**, *41*, 2289–2301.
4. De Lombaert, S.; Erion, M. D.; Tan, J.; Blanchard, L.; El-Chehabi, L.; Ghai, R. D.; Sakane, Y.; Berry, C.; Trapani, A. J. *J. Med. Chem.* **1994**, *37*, 498–511.
5. Atherton, F. R.; Hassall, C. H.; Lambert, R. W. *J. Med. Chem.* **1986**, *29*, 29–40.
6. (a) Fields, E. K. *J. Am. Chem. Soc.* **1952**, *74*, 1528–1531; (b) Walsh, E. N. *J. Am. Chem. Soc.* **1959**, *81*, 3023–3026; (c) Tyka, R. *Tetrahedron Lett.* **1970**, *9*, 677–680; (d) Qian, C.; Huang, T. *J. Org. Chem.* **1998**, *63*, 4125–4128.
7. (a) Tyka, R. *Tetrahedron Lett.* **1970**, *9*, 677–680; (b) Qian, C.; Huang, T. *J. Org. Chem.* **1998**, *63*, 4125–4128; (c) Heydari, A.; Karimian, A.; Ipaktschi, J. *Tetrahedron Lett.* **1998**, *39*, 6729–6732; (d) Ranu, B. C.; Hajra, A.; Jana, U. *Org. Lett.* **1999**, *1*, 1141–1143.
8. (a) Laschat, S.; Kunz, H. *Synthesis* **1992**, 90–95; (b) Smith, A. B.; Yager, K. M.; Taylor, C. M. *J. Am. Chem. Soc.* **1995**, *117*, 10879–10888.
9. (a) Campbell, D. A.; Bermak, J. C.; Burkoth, T. S.; Patel, D. V. *J. Am. Chem. Soc.* **1995**, *117*, 5381–5382; (b) Campagne, J.-M.; Coste, J.; Jouin, P. *Tetrahedron Lett.* **1995**, *36*, 2079–2082.
10. (a) Jiracek, J.; Yiotakis, A.; Vincent, B.; Checler, F.; Dive, V. *J. Biol. Chem.* **1996**, *271*, 19606–19611; (b) Burchardt, J.; Ferreras, M.; Krog-Jensen, Ch.; Delaisee, J.-M.; Foged, N. T.; Meldal, M. *Chem. Eur. J.* **1999**, *10*, 2877–2884.
11. Zhang, Ch.; Mjalli, A. M. M. *Tetrahedron Lett.* **1996**, *37*, 5457–5460.
12. Heydari, A.; Karimian, A.; Ipaktschi, J. *Tetrahedron Lett.* **1998**, *39*, 6729–6732.
13. Qian, Ch.; Huang, T. *J. Org. Chem.* **1998**, *63*, 4125–4128.
14. De Lombaert, S.; Stamford, L. B.; Blanchard, L.; Tan, J.; Hoyer, D.; Diefenbacher, C. G.; Wei, D.; Wallace, E. M.; Moskal, M. A.; Savage, P.; Jeng, A. Y. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1059–1064.
15. General solid-phase method used for preparation of a starting material **2** on MBHA resin (1.1 mmol/g): neutralization with 5% *N*,*N*′-diisopropylethylamine in DCM (10 ml/100 mg of resin, 2×2 min), wash with DCM (5×10 ml/100 mg of resin), condensation using 6 equiv. of an *N*-*tert*-butyloxycarbonyl (Boc) amino acid derivative, 6 equiv. of HOBt and 6 equiv. of diisopropylcarbodiimide in DMF (6 ml/100 mg of resin), deprotection with 55% trifluoroacetic acid in DCM (10 ml/100 mg of resin, 20 min), wash with DCM (6×10 ml/100 mg of resin); L-amino acid derivatives used: Boc-Ala, Boc-Asp (β-benzylester), Boc-Phe, Boc-Gly, Boc-Pro, Boc-Val.
16. The resin was treated with 0.5 M Me_3SiBr in DMF (6 ml of a solution per 100 mg of a resin) at rt overnight. Following washing with DMF, the formed silyl esters were hydrolyzed in water/DMF (1:5, 20 ml/100 mg of resin) at rt overnight.
17. Robl, J. A.; Duncan, L. A.; Pluscec, J.; Karanewsky, D. S.; Gordon, E. M.; Ciosek, C. P., Jr.; Rich, L. C.; Dehmel, V. C.; Slusarchyk, D. A.; Harrity, T. W. *J. Med. Chem.* **1991**, *34*, 2804–2815.
18. **4a**: ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.90–171.84, 166.52, 166.24, 137.85–137.07, 135.34–135.01, 133.28, 131.88, 129.23, 129.16, 129.02, 128.57–128.18, 127.76, 127.20–12.89, 126.54–126.41, 61.19–58.78, 54.51, 53.79, 37.57, 36.23, 35.44. **4b**: ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.99–172.84, 172.31, 172.08–172.67, 137.96–137.67, 129.27, 128.93, 128.29–128.10, 127.76–127.70, 127.05, 126.32–126.26, 59.71, 58.55, 55.68–55.57, 53.88–53.70, 37.90, 37.55, 37.22, 34.70. **4c**: ^1H NMR (500 MHz, DMSO-d_6): δ 8.24–8.23 (d, J=6.5 Hz 1H), 7.89 (br, 1H), 7.47–7.46 (d, J=6.2 Hz, 2H), 7.33–7.32 (m, 3H), 7.27–7.26 (m, 2H), 7.20–7.17 (m, 4H), 4.31–4.27 (m, 1H), 4.23 (s, 1H) 4.19 (s, 1H), 3.89 (br, 1H), 3.23 (br, 1H), 3.12–3.09 (dd, J=4.4 Hz, 4.0 Hz, 2H), 2.76–2.72 (m, 2H), 1.50 (br, 3H), 1.08 (br, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 173.27, 171.91, 137.58, 134.06, 130.35–130.29, 129.05–128.93, 128.29–128.01, 126.37, 64.64, 63.45, 54.12, 53.38, 37.58, 30.13, 23.33–23.19. ^{31}P (121.5 MHz, DMSO-d_6): δ 17.07 for one diastereomer and 18.51 for the other, in a 9:1 ratio, respectively.

19. (a) Rinnova, M.; Vidal, A.; Nefzi, A.; Houghten, R. A. *J. Comb. Chem.* **2002**, *43*, 2343–2346; (b) Vidal, A.; Nefzi, A.; Houghten, R. A. *J. Org. Chem.* **2001**, *66*, 8268–8272; (c) Rinnova, M.; Nefzi, A.; Houghten, R. A. *Tetrahedron Lett.*, submitted.
20. Redmore, D. *J. Org. Chem.* **1978**, *43*, 992–996.
21. A resin-bound dipeptide **2** was shaken with acetone (10 equiv.) and dimethyl phosphite (10 equiv.) in dry DCM (5 ml per 100 mg of resin) for 5 min. Then, 1 M $LiClO_4$ in dry diethyl ether (2 ml/100 mg of resin) was added and mixture was shaken for 16 h at rt (compound **7**: MS m/z 411.2; found: 411.9).