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The γ-Methyl-E-Olefin as Isosteric Replacement of the Peptide Bond

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Abstract: Different olefination methods on Boc-phenylalanine methyl ketone for the synthesis of γ-methyl-E-olefin Phe-Gly isosteres are investigated. The Horner phosphonate reagent gave the highest yield of E-isomer. The conformational behaviour of four tetrapeptides containing the E-olefin isostere with and without a vinyl methyl group is studied by ^{1}H NMR. No evidence for turn structures is found.

INTRODUCTION

In many biologically active peptides the C=C double bond isostere has proven to be a succesful replacement of proteolytically susceptible peptide bonds. The electronic and steric characteristics of these E-olefin isosteres are however quite different from those of an amide function. The fluoro olefin isostere has been used as a better mimic with an increased polarity and a restored hydrogen bonding capacity.[1,2] Semi-empirical energy calculations of model alkene dipeptide isosteres have indicated an increased flexibility compared to the parent peptide as a result of the replacement of the amide oxygen by the smaller hydrogen atom.[3,4] These calculations also predict the methylsubstituted alkene, in which the methyl simulates the steric influence of the oxygen, to show an increased rigidity and a strong β I turn forming tendency.[3]

We now report the synthetic studies for the preparation of the γ-methyl-E-olefin isostere and a conformational study by NMR to compare the structure of alkene and methylalkene model tetrapeptides.

SYNTHESIS

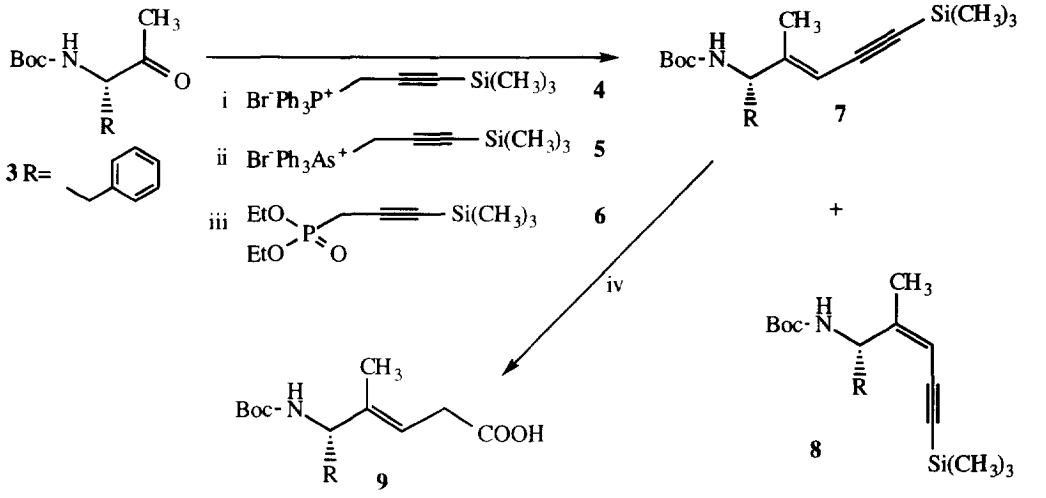
Many different synthetic pathways for E-olefinic pseudodipeptides exist in the literature.[5]

The method described by Hann et al is used to prepare Boc-PheΨ(E,CH=CH)Gly-OH **1** starting from N-Boc-Phe. The procedure, using a Wittig olefination reaction, followed by hydroboration and oxidation of the resulting enyne, is however not racemization free and the enantiomeric excess (52 %) of **1** is determined by derivatization of the aminofunction with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC).[6] The pseudodipeptide **1** is incorporated as a D,L-mixture in the tetrapeptide sequence Ac-Ala-D,L-PheΨ(E,CH=CH)Gly-Ala-NH_2 **2** by solid phase peptide synthesis on a 4-methylbenzhydrylamine resin using Boc protected amino acids and diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazool (HOBt) as coupling reagents. The resin bound peptide is N-acetylated by treatment with Ac_2O and triethylamine in DMF. Cleavage from the resin using HF_{liq} and anisole as scavenger yields the amide **2**. The diastereoisomers are separated by HPLC at this stage.

The preparation of γ-alkylated E-olefin dipeptide isosteres involving an asymmetric aldol condensation and subsequent stereospecific [3,3] rearrangement has been recently reported.[7] We have studied the olefination

of the methylketone **3**, prepared from Boc-Phe [8], using three alternative procedures: a Wittig reaction using a phosphonium salt **4** [6] or an arsonium salt **5** [9] and a Horner-Wadsworth-Emmons reaction using diethyl-(3-trimethylsilyl-2-propynyl)-phosphonate **6** [10] (scheme 1).

Scheme 1



Conditions: i. n-BuLi (1.6 M in hexane), anhydrous THF, -40°C, 3 h; then **3** at -75°C, 30 min and at 20°C, 24.5 h. ii. n-BuLi (1.6 M in hexane), anhydrous THF, -50°C, 2h; then **3** at -70°C, 30 min and at 20°C, 20 h. iii. $NaN(Si(CH_3)_3)_2$, anhydrous THF, -60°C, 25 min; then **3** at -60°C, 20 min and at 20°C, 50 min. iv. $(C_6H_{11})_2BH$ (see footnote 11), anhydrous THF, 20°C, 3 h; then CH_3OH, 3N NaOH, 30 % aq H_2O_2, 0°C, 30 min.

Reaction with the phosphonium ylid gives an excellent E/Z selectivity, but a low yield. The chemical yield is improved with the arsonium ylid, but with loss of selectivity, so that the yield of isolated **7** drops to 15 %. Reaction with the phosphonate **6** gives the best yield with an acceptable selectivity, resulting in a 46 % isolated yield of the E enyne **7**. The shorter reaction time of the phosphonate carbanion (50 min) compared to the phosphonium (24 hours) or arsonium (20 hours) ylids confirms its increased nucleophilicity (Table 1).[10]

Table 1 Yields of the different Olefination Reactions and Enantiomeric Excess of the final E Olefinic Compound **9**.

carbanion of	yield **7** + **8** (%)	**7**/**8**	yield **7** (%)	ee (%) **9**
4	24	93/7	22	38
5	35	42/58	15	-
6	57	81/19	46	48

The E and Z enynes **7** and **8** are separated by HPLC (Lichrosorb SiO_2, CH_2Cl_2). The E isomer is distinguished from the Z isomer by the chemical shift of the methyl signal which is low field in the ^1H NMR spectrum [12] (1.92 ppm in **7**, 1.67 ppm in **8**) and high field in the ^{13}C NMR spectrum (16.89 ppm in **7**, 22.69 ppm in **8**). After hydroboration with dicyclohexylborane at 20°C and oxidation using H_2O_2 in basic aqueous medium at 0°C, the enyne **7** is converted to the δ-amino acid **9** with 56 % yield. Racemization occurs during all olefination procedures. The enantiomeric excess, 38 % after Wittig and 48 % after Horner-Wadsworth-Emmons olefination, is again determined by the GITC method.[6] For the incorporation of this D,L-mixture of **9** into the

tetrapeptide sequence the same solid phase peptide synthesis procedure as described above is used. The Ac-Ala-D,L-PheΨ(E,C(CH_3)=CH)Gly-Ala-NH_2 **10** diastereomers are separated by HPLC. The conformation of both the L- **L10** and D-Phe pseudopeptides **D10** are investigated by ^1H NMR spectroscopy, since the D residue at position (i+1) may have a favourable effect on the formation of a β II' turn.[13]

NMR

1D and 2D ROESY[14] and HOHAHA[15] ^1H NMR spectra in DMSO-d_6 (99.96% D, Aldrich) are recorded to investigate the possibility of β-type turn formation. A small temperature dependence of the Ala^4 NH, i. e. a value smaller than 2 ppb/K, would be indicative of a H bond stabilizing such a turn. Furthermore β turns are characterized by short interproton distances of the type d_{NN}(i+1, i+2) (in a βI turn), $d_{\alpha N}$(i+1, i+2) (in a βII turn), $d_{\alpha N}$(i+1, i+3) and d_{NN}(i+2, i+3) (in both βI and βII turns).[13] The $d_{\alpha N}$(i+2, i+3) type nOe's are absent in β turns. In these peptides the role of the NH of residue i+2 (i. e. Gly^3) is played by a vinyl H. As shown in Table 2, none of these characteristics were found.

Table 2. Temperature Coefficients of NH Protons (ppb/K), Vicinal Coupling Constants (Hz) and relative Intensities of ROESY Cross Peaks (+++: strong, ++: medium, +: weak, -: absent) of L2, D2, L10, D10 in DMSO. Only Data relevant to the Conformation are given.

residue		L2	D2	L10	D10
i: Ala^1	$-\Delta\delta/\Delta T$ (NH)	5.7	*	6.0	6.3
	$^3J_{NH\text{-}C\alpha H}$	7.2	**	7.7	7.5
i+1: ψPhe^2	$-\Delta\delta/\Delta T$ (NH)	6.5	*	7.0	7.3
	$^3J_{NH\text{-}C\alpha H}$	8.0	8.7	8.4	8.4
	d_{NCH}(i+1,i+2)	-	-	+	++
	$d_{\alpha CH}$(i+1,i+2)	-	-	+++	+++
	$d_{\alpha N}$(i+1,i+3)	-	-	-	-
i+2: ψGly^3	d_{CHN}(i+2,i+3)	-	-	-	-
	$d_{\alpha N}/d_{\alpha' N}$	++/++	-	+++/-	**
i+3: Ala^4	$-\Delta\delta/\Delta T$ (NH)	7.4	*	7.3	8.4
	$^3J_{NH\text{-}C\alpha H}$	7.5	**	7.6	7.4

Notes: * No temperature study is performed.
** Overlap.

The vicinal coupling constants, the small number of nOe cross peaks in the ROESY and the rather large values of temperature dependence of the amide proton chemical shifts indicate a flexible structure with no preferred backbone conformation and no intramolecular H bonds.

CONCLUSION

The similarity between the spectra of the pseudopeptides with and without a γ CH_3 does not allow to detect any difference in conformational rigidity nor preference in these short tetrapeptides. The results of the previous theoretical studies could not be confirmed. The synthetic approach using either a Wittig or a Horner-Wadsworth-Emmons olefination reaction suffers mainly from extensive racemization. Since, compared to other

amino-acids, phenylalanine is most sensitive to racemization [5], the method might however be useful for other residues.

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REFERENCES AND NOTES

1. Abraham, R. J.; Ellison, S. L. R.; Schonholzer, P.; Thomas, W. A. *Tetrahedron* **1986**, 42, 2101-2110.
2. Allemendinger, T.; Furet, P.; Hungerbühler, E. *Tetrahedron Lett.* **1990**, *31*, 7297-7300.; Allemendinger, T.;Felder, E.; Hungerbühler, E. *Tetrahedron Lett.* **1990**, 31, 7301-7304.
3. Deschrijver, P.; Tourwé, D. *FEBS Lett.* **1982**, 146, 353-356.
4. Baginski, M.; Piela, L.; Skolnick, J. *J. Comput. Chem.* **1993**, 14, 471-477.
5. Cox, M. T.; Gormley, J. J.; Hayward, C. F.; Petter, N. N. *J. Chem. Soc. Chem. Commun.* **1980**, 800-802.; Hann, M. M.; Sammes, P. G.; Kennewell, P. D.; Taylor, J. B. *J. Chem. Soc. Perkin Trans. 1* **1982**, 307-314.; Miles, N. J.; Sammes, P. G.; Kennewell, P. D.; Westwood, R. *J. Chem. Soc. Perkin Trans. 1* **1985**, 2299-2305.; De Cock, E.; Van Marsenille, M.; Van der Auwera, L.; Tourwé, D.; Van Binst, G. *Tetrahedron* **1986**, 42, 5403-5406.; Spaltenstein, A.; Carpino, P. A.; Miyake, F.; Hopkins, P. B. *Tetrahedron Lett.* **1986**, 27, 2095-2098.; Shea, R. G.; Fitzner, J. N.; Fankhauser, J. E.; Spaltenstein, A.; Carpino, P. A.; Peevey, R. M.; Pratt, D. V.; Tenge, B. J.; Hopkins, P. B. *J. Org. Chem.* **1986**, 51, 5243-5252.; Spaltenstein, A.; Carpino, P. A.; Miyake, F.; Hopkins, P. B. *J. Org. Chem.* **1987**, 52, 3759-3766.; Shue, Y.-K.; Carrera, G. M., Jr.; Nadzan, A. M. *Tetrahedron Lett.* **1987**, 28, 3225-3228.; Lehman de Gaeta, L. S.; Czarniecki, M.; Spaltenstein, A. *J. Org. Chem.* **1989**, 54, 4004-4005.; Whitesell, J. K.; Lawrence, R. M. *Chirality* **1989**, 1, 89-91.; Shue, Y.-K.; Carrera, G. M., Jr.; Tufano, M. D.; Nadzan, A. M. *J. Org. Chem.* **1991**, 56, 2107-2111.; Ibuka, T.; Habashita, H.; Otaka, A.; Fujii, N.; Oguchi, Y.; Uyehara, T.; Yamamoto, Y. *J. Org. Chem.* **1991**, 56, 4370-4382.; Fujii, N.; Habashita, H.; Shigemori, N.; Otaka, A.; Ibuka, T.; Tanaka, M.; Yamamoto, Y. *Tetrahedron Lett.* **1991**, 32, 4969-4972.; Bol, K. M.; Liskamp, R. M. J. *Tetrahedron Lett.* **1991**, 32, 5401-5404.; Bol, K. M.; Liskamp, R. M. J. *Tetrahedron* **1992**, 48, 6425-6438.; Bohnstedt, A. C.; Vara Prasad, J. V. N.; Rich, D. H. *Tetrahedron Lett.* **1993**, 34, 5217-5220.; Beresis, R.; Panek, J. S. *Bioorg. Med. Chem. Lett.* **1993**, 3, 1609-1614.; Jenmalm, A.; Berts, W.; Li, Y.-L.; Luthman, K.; Csöregh, I.; Hacksell, U. *J. Org. Chem.* **1994**, 59, 1139-1148.; Wipf, P.; Fritch, P. C. *J. Org. Chem.* **1994**, 59, 4875-4886.; McKinney, J. A.; Eppley, D. F.; Keenan, R. M. *Tetrahedron Lett.* **1994**, 35, 5985-5988.; Ibuka, T.; Nakai, K.; Habashita, H.; Hotta, Y.; Fujii, N.; Mimura, N.; Miwa, Y.; Taga, T.; Yamamoto, Y. *Angew. Chem. Int. Ed. Engl.* **1994**, 33, 652-654.
6. Devadder, S.; Couder, J.; Jaspers, H.; Ceusters, M.; Tourwé, D.; Van Binst, G. *Bull. Soc. Chim. Belg.* **1991**, 100, 407-409.
7. Wai, J. S.; Fisher, T. E.; Embrey, M. W. *Tetrahedron Lett.* **1995**, 36, 3461-3464.
8. Kawai, M.; Boparai, A. S.; Bernatowicz, M. S.; Rich, D. H. *J. Org. Chem.* **1983**, 48, 1876-1880.
9. Shen, Y.; Liao, Q. *J. Organomet. Chem.* **1988**, 346, 181-184.
10. Gibson, A. W.; Humphrey, G. R.; Kennedy, D. J.; Wright, S. H. B. *Synthesis* **1991**, 414-416.
11. $(C_6H_{11})_2BH$ is in situ prepared from cyclohexene and $BH_3.S(CH_3)_2$ in THF at 0°C.
12. Jackman, L. M.; Wiley, R. H. *J. Chem. Soc.* **1960**, 2881-2882.
13. Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, 37, 1-97.
14. Bothner-By, A. A.; Stephens, R. L.; Lee, J. M. *J. Am. Chem. Soc.* **1984**, 106, 811-813.
15. Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, 65, 355-360.

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