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Determination of solute descriptors of tripeptide derivatives based on high-throughput gradient high-performance liquid chromatography retention data[1]

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Abstract

Recently proposed chromatographic hydrophobicity indices (CHIs) have been calculated from the correlation of fast gradient HPLC retention times with φ_0 values from isocratic HPLC measurements for 30 oligopeptide derivatives. Measurements were performed on five HPLC columns; an Inertsil ODS (In), a Prodigy ODS (Pro), an immobilised artificial membrane (IAM), a permethylated β-cylcodextrin (CD) and a cyanopropyl (CN) stationary phase. The ranking of CHI values of the tripeptide derivative of the type Z–Ala–Xaa–Val–OMe for the CD, IAM and CN phases is comparable with other amino acid hydrophobicity scales. The CHI values were used for the determination of three molecular descriptors of the solvation equation established by Abraham; these are the effective hydrogen-bond acidity $\Sigma\alpha_2^H$, the effective hydrogen-bond basicity $\Sigma\beta_2^H$ and the solute dipolarity/polarisability π_2^H. The comparison of the solute descriptors of the tripeptide derivatives in terms of the change of the sequence and the chirality of the amino acids shows a strong influence on $\Sigma\alpha_2^H$, $\Sigma\beta_2^H$ and π_2^H.

Keywords: Hydrophobicity indices; Molecular descriptors; Peptides

1. Introduction

The hydrophobicity/lipophilicity of a compound is an important parameter in medical, pharmaceutical and environmental chemistry. In the field of peptide and protein research the significance of hydrophobic interactions as a determining parameter for the folding processes has been appreciated since Kauzmann's review in 1959 [1]. Usually the hydrophobicity/lipophilicity is quantitatively characterised as log P values where P is a water–solvent partition coefficient. In this work we will use the term hydrophobicity in order to describe solute properties like log P and the capacity factor log k'. The extensive work of Hansch and coworkers [2,3] and of Leo et al. [4] established water–octanol as the preferred system. Although the traditional experimental method for the determination of log P_{oct} is the shake-flask method this procedure is quite time-consuming and requires pure, aqueous soluble

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[1]Dedicated to Professor A. Kolbe on the occasion of his 65th birthday.

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samples and usually higher amounts of substance in mg quantities. Much effort has been put into the testing of more efficient methods. For example correlation between log P_{oct} and the capacity factor k' obtained from reversed-phase high-performance liquid chromatography (RP-HPLC) measurements has shown that log k' or log k_0 values can be used as hydrophobicity parameters. However, certain precautions must be taken when using log k' or log k_0 [5] because the solute factors which influence log P_{oct} are not the same as those which influences log k' or log k_0 [6]. Therefore, Valko and Slegel [7] introduced a new chromatographic hydrophobicity index φ_0 which can be calculated from log k_0 and includes the slope of the regression line of the log k' vs. % organic modifier. For the determination of φ_0 for a compound, measurements of the retention factors at several solvent compositions are necessary. Thus, for industrial needs where high throughput technologies are demanded even these isocratic RP-HPLC measurements are not fast enough. Recently, Valko et al. [8] reported a new chromatographic hydrophobicity index (CHI) based on fast gradient HPLC measurements which can be used as an alternative to log P_{oct}. Furthermore, it was shown that this CHI value can be used as a solute related property in quantitative structure–property relationship (QSPR) analysis with sufficient precision [9].

Among other QSPRs the general equation

$$\log SP = c + rR_2 + s\pi_2^H + a\sum \alpha_2^H + b\sum \beta_2^H + \nu V_x \quad (1)$$

established by Abraham [10] describes the linear relationship between a property of a set of solutes in a given solvent system (SP) and the explanatory variables, or descriptors of the solute. Therein R_2 is the excess molar refraction and π_2^H the solute dipolarity/polarisability. The parameters $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ describe the effective hydrogen-bond acidity and basicity, respectively. V_x is the McGowan characteristic volume in units of $dm^3\ mol^{-1}/100$ that can be calculated for any solute from the molecular structure, using the atomic volumes and bond constants [11] and the algorithm of Abraham for the number of bonds in any molecule [10]. Also the excess molar refraction can be calculated [12,13]. The determination of $\Sigma\alpha_2^H$, $\Sigma\beta_2^H$ and π_2^H descriptors can be usually done from partition coefficients measured in different organic–water solvents [14,15].

In this paper we will present the first attempts to use the CHI value which is obtained from fast gradient HPLC measurements in less than 10 min as a solute related property in QSPR equations for the calculation of descriptors for peptide derivatives. Considering the importance of high-throughput methods for industrial application, the use of the CHI values for the determination of the solute descriptors would give a challenging impulse for the discovery of new drug molecules.

The systematic study of solute descriptors of tripeptide derivatives will indicate how the hydrophobicity alters with change of composition, sequence and chirality of the peptide.

2. Experimental

2.1. Substances

The tripeptide and dipeptide derivatives were synthesised by the group of C. Griehl (Institute of Organic Chemistry, Martin-Luther-University, Halle, Germany); their physical characteristics are in part described elsewhere [16]. Ac–Phe–OMe was purchased from Advanced ChemTech (Louisville, KY, USA). In order to avoid any kind of head–tail interactions all substances were protected at the *N*- and *C*-terminal functions. The Z (benzyloxycarbonyl), Ac (acetyl) and the Boc (*tert*.-butyloxy-carbonyl) residues were used for the protection of the *N*-terminal site. The acidic function was blocked by ester residues which were either methyl (OMe), ethyl (OEt), *tert*.-butyl (OtBu) or benzyl (OBzl) esters.

2.2. Calculation

V_x was calculated with a software routine written by Abraham and Roses [17]. The calculation of the excess molar refraction R_2 is based on the refractive index. However, all our compounds are solid at room temperature, but since the refractive index in terms of molar refraction is an additive property it can be approximated from substructure. For the calculation of the refractive index based on the group contribu-

Table 1
Details of the commercially available columns used in this study

Name	Dimension	Supplier	Abbreviation
ODS2-IK5 Inertsil	5 μm, 150×4.6 mm	A	CHI_{In}
Prodigy ODS2	5 μm, 150×4.6 mm	B	CHI_{Pro}
Novapak-CN	4 μm, 75×3.9 mm	C	CHI_{NCN}
RexChrom IAM PC2	12 μm, 150×4.6 mm	D	CHI_{IAM}
Nucleodex β PM	5 μm, 200×4.6 mm	E	CHI_{CD}

Suppliers: A=Capital HPLC, Broxburn, UK; B=Phenomenex UK, Macclesfield, UK; C=Waters Chromatography, Watford, UK; D=Pierce and Warriner (UK), Chester, UK; E=Fisher Scientific UK, Loughborough, UK.

tion of atoms and structural groups to the refractive index we used the ACD/CHEM SKETCH software. The multiple regression analysis has been carried out using the DRUGIDEA software package (Chemicro, Budapest, Hungary) and with the SMARTWARE II (Informix Software).

2.3. HPLC measurement

A Hewlett-Packard 1090 series HPLC system was used. Data acquisition and processing were performed on a Viglen IBM compatible personal computer with HP CHEMSTATION software (Hewlett-Packard, Amsterdam, Netherlands) using a 0.05 mol/l ammonium acetate buffer (pH 7.4) as the mobile phase. HPLC grade acetonitrile (ACN; Rathburn, Walkerburn, UK) was used as organic modifier.

The applied chromatographic columns are shown in Table 1.

A flow-rate of 1.5 ml/min was used. For the CD column the flow-rate was 1.0 ml/min in the isocratic experiment. Ten μl of each substance which was dissolved in an acetonitrile–buffer (0.001 mol/l) (1:1) were injected. The temperature was kept constant at 26°C. Isocratic measurements were performed changing the composition of the mobile phase systematically. The concentration of acetonitrile was increased in 5% steps. The elusion of the peptides took less than 10 min.

For the fast gradient retention time measurements the composition of the mobile phase was changed from 0 to 100% of acetonitrile within 3.5 min; great care was taken in order to guarantee the complete recovery of the stationary phase. Thus, following the gradient experiment a washing process of 1 min with 100% acetonitrile and 2.3 min with the pure buffer solution was carried out.

2.4. Calculation of φ_0 and CHI

The correlation of the CHI values was based on the retention times of a standard mixture containing seven alkylarylphenones. The isocratically determined φ_0 values (see Table 2) measured specifically

Table 2
Isocratically determined φ_0 values for the standard mixture for the five different columns

Test compound	$\varphi_{0,CN}$	$\varphi_{0,IAM}$	$\varphi_{0,CD}$	$\varphi_{0,Pro}$	$\varphi_{0,In}$
Theophylline	–	−1.93	−6.25	–	–
Paracetamol	−1.85	2.95	11.84	6.26	−1.93
Acetanilide	4.72	11.48	32.75	42.45	37.94
Acetophenone	15.36	17.15	46.91	64.01	61.33
Propiophenone	25.48	25.88	53.40	74.39	72.31
Butyrophenone	32.79	32.04	56.67	81.25	79.24
Valerophenone	38.53	37.33	60.41	86.67	85.00
Hexanophenone	42.16	41.82	64.22	91.23	89.86
Heptanophenone	44.12	45.65	67.54	95.71	94.67
Octanophenone	46.11	49.37	70.52	99.72	99.28

for each column were linearly correlated with the retention times t_R obtained from the fast gradient measurements according to the approach described in [8].

The results of the linear regression of φ_0 vs. retention time t_R of the standard mixture were used for the calculation of the CHI values of the peptides based on their retention times according to: $\varphi_0 = At_{Rs} + B$ (standard) and $CHI_x = At_{Rx} + B$ (for sample x).

For the calculation of the solute descriptors the specific column parameters which were obtained from the CHI values of 29 test compounds with known descriptors were taken from Ref. [9]. From 20 different RP-HPLC systems presented in that paper we chose those five columns with the most different coefficients *s*, *a* and *b* for the determination of π_2^H, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$. The coefficients of the columns as documented in the above mentioned paper are given in Table 3.

The CHI values were then used as solute related properties in Eq. (1). We then have five equations of the type of Eq. (1) that contain the coefficients given in Table 3. The descriptors R_2 and V_x can be calculated for any peptide and so we are left with three descriptors to determine ($\Sigma\alpha_2^H$, $\Sigma\beta_2^H$ and π_2^H) using five equations. A fitting procedure was then used to find the best solutions for the three descriptors.

3. Results and discussion

3.1. Calculation of the CHI values

Table 4 shows the CHI values for a selection of oligopeptides with the general formula Z–Ala–Xaa–Val–OMe where the second amino acid residue was varied. Table 4 indicates clearly that small structural changes in the tripeptide derivative shows up in their CHI values; the CHI value (CD column) for Z–Ala–Leu–Val–OMe is 38.6 and for Z–Ala–Val–Val–OMe is 35.9, respectively. Thus, the addition of an CH_2 group in the side chain leads – as expected – to an increase of the hydrophobicity of the entire tripeptide molecule. However, the change of the sequence from Z–Ala–Phe–Val–OMe to Z–Ala–Val–Phe–OMe results in a small decrease of the CHI values. Thus, the formation of secondary structures influences the elution order e.g., hydrophobicity as well.

The effect of the diastereoismerism is very small. In general, we calculated smaller CHI values for the LLL diastereoisomers than for the LDL analogue. However, the percentage of the deviation between both CHI values depends on the amino acid Xaa. A substitution of a L- amino acid by a D-amino acid at the *N*-terminal end of the tripeptide derivative as shown on the examples, Z–Ala–Phe–Val–OMe and Z–Ala–Leu–Val–OMe in Table 4 has, however, no

Table 3
Results of the multiple regression using the CHI parameter as solute-related property according to Ref. [9]

Column	Overall correlation	S.E.	*r*	*s*	*a*	*b*	*v*	*c*
IAM	0.972	3.4	10.2 ±2.7	−11.0 ±2.9	6.5 ±2.8	−47.4 ±3.5	44.0 ±2.4	0.7
CD	0.970	4.9	7.5 ±3.8	−4.2[a] ±4.2	−1.9[a] ±4.0	−52.0 ±5.0	31.5 ±3.4	36.5
CN	0.957	5.3	9.0 ±4.1	−13.1 ±4.5	−7.1 ±4.2	−30.0 ±5.4	48.8 ±3.7	−18.0
Pro	0.993	3.0	3.4 ±2.3	−12.4 ±2.5	−23.2 ±2.4	−61.9 ±3.05	58.1 ±2.1	39.8
In	0.987	4.5	5.9 ±1.8	−15.3 ±2.0	19.2 ±1.9	−63.7 ±2.4	65.0 ±1.6	28.6

[a] Statistically not significant variable; the standard error (S.E.) of the estimate is given beneath each coefficient.

Table 4
CHI values of selected amino acid, dipeptide and tripeptide derivatives

Peptide	CHI_{CD}	CHI_{IAM}	CHI_{CN}	CHI_{Pro}	CHI_{In}
Z–Ala–**Trp**–Val–OMe					
LLL	44.91	28.86	44.44	64.18	61.10
LDL	45.78	29.01	44.52	64.84	61.80
Z–Ala–**Phe**–Val–OMe					
LLL	41.85	25.87	43.95	67.09	63.75
LDL	42.65	26.10	43.44	65.63	65.54
DLL	42.61	26.04	43.96	67.29	64.04
Z–Ala–**Leu**–Val–OMe					
LLL	**38.60**	23.32	41.92	65.98	61.93
LDL	38.94	24.44	42.16	67.95	63.99
DLL	38.79	23.74	41.89	66.44	62.52
Z–Ala–**Tyr**–Val–OMe					
LLL	37.51	23.66	38.98	54.47	50.63
LDL	38.12	24.32	39.21	54.81	50.81
Z–Ala–**Val**–Val–OMe					
LLL	**35.90**	20.49	38.37	60.99	56.64
LDL	36.53	22.72	39.47	62.86	59.26
Z–Ala–**His**–Val–OMe					
LLL	28.81	18.43	35.73	42.05	37.76
LDL	74.48	30.65	34.91	60.50	62.22
Z–Ala–**Ser**–Val–OMe					
LLL	25.98	17.62	30.67	45.56	41.01
LDL	26.68	18.47	31.39	45.88	41.44
Z–Ala–**Asn**–Val–OMe					
LLL	23.62	16.62	30.49	42.08	37.65
LDL	23.89	17.18	30.87	42.05	37.58
Z–Ala–**Tyr(OBzl)**–Val–OMe					
LLL	50.34	32.25	48.81	77.11	76.22
LDL	51.29	32.60	49.23	78.86	77.45
Z–Ala–**Lys(NBzl)**–Val–OMe					
LLL	44.22	26.58	45.37	66.97	64.40
LDL	47.28	29.97	46.50	73.89	72.07
Z–Ala–**Asp(OBzl)**–Val–OMe					
LLL	44.19	27.67	45.46	70.57	68.50
LDL	44.83	27.91	45.61	71.20	69.22
Z–Ala–**Ser(OBzl)**–Val–OMe					
LLL	42.95	27.82	44.83	70.06	67.76
LDL	43.54	27.85	44.98	70.64	68.44
Ac–Phe–OMe	25.00	9.88		44.03	36.99
Z–Ala–Val–OMe	36.51	21.08	33.38	58.92	55.47
Z–Ala–Val–Phe–OMe	41.60	26.07	42.58	64.72	62.18
Z–Ala–Val–Leu–OMe					
LLL	38.79	23.52	41.62	66.12	62.06
LDL	39.09	25.22	42.16	68.04	64.20

effect on the CHI value. For RP phases Aguilar et al. [18] reported similar observations on the retention behaviour of neuropeptide analogues with differing amino acid chirality.

We would like to remark that the different CHI values for LLL and LDL diastereoisomers were not only found on the RP phases but also on the permethylated β-cyclodextrin and on the immobilised artificial membrane as well. This is surprising because the separation mechanism should be different in the last mentioned columns. In cyclodextrin phases the separation of molecules is based primarily on the cavity formation of the cyclodextrin matrix in which the molecules or parts of them were incorporated. The strength of the interaction on the outer sphere of those cavities depends on the ability of the molecules to form hydrogen bonds [19]. In contrast, the separation on immobilised artificial membranes depends on the strength of the interaction between the peptide and the hydrophobic artificial membrane surface [20]. Thus, we would conclude that despite the different separation mechanism the main influence on the retention time in our gradient experiments is still the partition of the peptides between the apolar groups on the stationary phase and the polar mobile phase. Additionally, the separation of the diastereoisomers is aided by specific hydrogen bond interactions of the molecules with the polar functions on the CD and IAM column.

Hydrophobicity scales of amino acids have been described in various papers in the literature [21–32]. For a general overview the paper of Wilce et al. [29] is recommended. However, the ranking of the amino acids is not the same as in various scales and depends strongly on the chosen peptides and the experimental conditions. Thus, the number and kind of amino acids and their sequence in the proteins, on the column material, and on the solvent composition influences the ranking of the 20 essential amino acids [29–33].

In our case the set of LLL diastereoisomers of tripeptides is too small for a comprehensive analysis of the influence of each amino acid residue. However, we can compare the ranking of our CHI values with literature scales for peptides with the uniform structure Z–Ala–Xaa–Val–OMe, since Xaa should be responsible for the differences in CHI (Table 4). Table 5 gives the ranking of the Xaa amino acids we

Table 5
Ranking of amino acid hydrophobicity scales Z–Ala–**Xaa**–Val–OMe in comparision to literature data

Xaa	a	**b**	c	**d**	e	f	g	CHI_{Pro}	CHI_{In}	**CHI_{IAM}**	**CHI_{CD}**	**CHI_{CN}**
Trp	16	**1**	1	**1**	1	6	3	3	3	**1**	**1**	**1**
Phe	3	**3**	3	**2**	3	5	5	1	1	**2**	**2**	**2**
Leu	1	**4**	4	**3**	2	12	11	5	5	**4**	**3**	**3**
Tyr	2	**8**	5	**5**	7	7	19	2	2	**3**	**4**	**4**
Val	6	**7**	8	**7**	6	14	6	4	4	**5**	**5**	**5**
His	10	**12**	16	**14**	11	2	20	7	7	**6**	**6**	**6**
Ser	17	**14**	10	**19**	10	9	7	6	6	**7**	**7**	**7**
Asn	19	**16**	12	**20**	19	11	4	8	8	**8**	**8**	**8**

Ranking according to ascendant hydrophobic amino acid.

a: Derived hydrophobicity coefficients for each amino acid based on solubility data (ethanol–water partition energies) [22].

b: Based on the octanol–water amino acid partitioning data of all 20 amino acids [33].

c: Used regression data analysis of RP-HPLC retention times of 25 peptides. Mobile phase, $NaClO_4$–ACN–water (gradient elution) pH 7; sorbent, RP-18 [34].

d: Extended version of c examined the retention time of 100 peptides. Mobile phase, $NaClO_4$–ACN–water (gradient elution) pH 7; sorbent, RP-18 [31].

e: Based on the retention times of 20 synthetic peptides with specifically substituted amino acids, and calculated the contributions of each of the 20 naturally occuring amino acid to the retention times. Mobile phase, TFA–ACN–water (gradient elution) pH 7; sorbent, RP-18 [35].

f: Based on the retention times of 1738 peptides (up to 50 AS units), and calculated the contributions of each of the 20 naturally occuring amino acid to the retention times. Mobile phase, TFA–ACN–water (gradient elution) pH 7; sorbent, RP-18 [30].

g: Based on the retention times of 1738 peptides (up to 50 AS units); the contributions contribution of each of the 20 naturally occurring amino acid to the retention times were calculated. Mobile phase, 0.1% TFA–2-PrOH–ACN–water (2-PrOH–ACN, 33:67) (gradient elution) pH 7; sorbent, RP-18 [30].

have used according to the various hydrophobicity scales. Note that although these scales include all 20 amino acids, we give rankings only for the particular amino acids we have used. Scales (b), (c), (d) and (e) in Table 5 give a rank order of Xaa very similar to the rank order we found on the five HPLC systems.

In particular, our scales of the IAM, CN and CD stationary phases represent the same ranking for the hydrophobicity of the amino acids. They fit best with the literature scales deduced from partitioning measurements in octanol–water of the amino acids (b) and other ODS data (d). The deviations found for the ODS columns (Pro and In) might be caused by the pocket formation on these columns [24] or adsorption/desorption effects [35].

Thus, we can conclude that the CHI value calculated from retention times in fast gradient HPLC can be used to express the hydrophobicity of the amino acids in a comparable array as the ''traditional'' methods and can be used for more detailed studies of peptide derivatives.

3.2. Calculation of the solute descriptors

As mentioned in Section 3.1 for CHI values of rather similar compounds, we found that small structural changes in the tripeptide derivatives can affect the hydrophobic character of the compounds. To get a deeper insight into these structural differences we analysed the CHI values in terms of the solute descriptors of Abraham, according to Eq. (1). This equation seems to be especially useful because it combines various properties of fundamental interactions like electrostatic forces (molar excess refraction) dipolar interactions (dipolarity/polarisability) and hydrogen bond interactions (acidity and basicity). The descriptors for the amino acid, dipeptide and tripeptide derivatives are given in Tables 6–8.

In the last row of Tables 7 and 8 the standard deviation (S.D.) of errors (in CHI units) is shown. In general, the error was less than 2 CHI units. Exceptions were only found in the case of the Lys(NBzl) and D-His. We assume that these compounds might ionise or be adsorbed on the stationary phase. Therefore, we exclude these values from further discussion. In order to determine useful descriptors for those compounds other stationary phases or pH dependent measurements should be tested.

Table 6
Comparision of the calculated descriptors with descriptors of selected compounds from the UCL database

Compound	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	π_2^H	R_2	$V_x/100$
Ac–Phe–OMe	0.00	1.34	1.78	1.514	1.752
Z–Ala–Val–OMe	0.24	1.50	3.14	0.956	2.630
Z–Ala–Phe–Val–OMe	0.09	1.96	6.12	1.807	3.776
Z–Ala–Val–Phe–OMe	0.16	1.94	6.26	1.807	3.776
Z–Ala–Leu–Val–OMe	0.22	1.88	5.28	1.096	3.591
Z–Ala–Val–Leu–OMe	0.22	1.87	5.31	1.096	3.591
n-Hexacosane	0	0	0	0	3.772
Ethanol	0.37	0.48	0.42	0.246	0.3082
Phenol	0.6	0.3	0.89	0.805	0.7751
Diethylamine	0.08	0.69	0.30	0.154	0.772
Urea	0.50	0.90	1.00	0.5	0.4648
Methylurethane	0.24	0.61	0.82	0.263	0.8464
N-Methylacetamide	0.40	0.72	1.30	0.4	0.6468
Acetanilide	0.5	0.67	1.4	0.87	1.1133
Ethyl acetate	0	0.45	0.62	0.106	0.7466
Acetophenone	0	0.48	1.01	0.818	1.0139
Theophylline	0.54	1.34	1.60	1.50	1.2223
Progesterone	0	1.14	3.29	1.45	2.6215

The precision of the descriptors π_2^H, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ themselves are strongly determined by the range of the coefficients of the chromatographic system given in Table 3: the larger the range of the coefficients, the smaller the deviation in the descriptor of the tripeptide derivative. Thus, we can give a precision of $\Sigma\beta_2^H$ with ± 0.01 units whereas the S.D. is larger for π_2^H and $\Sigma\alpha_2^H$ being ± 0.02 and ± 0.05, respectively. From the definition of the descriptors the values cannot become negative. Thus, the negative $\Sigma\alpha_2^H$ value for Trp shows the limitation of the method with regard to this descriptor. The search for new column systems in order to obtain a greater variety of coefficients in the equations is still in progress.

3.2.1. Comparison with other solutes (Table 6)

In Table 6 the descriptors for some simple solutes [36] for comparison with our data on peptides are shown. As expected, the peptides in Table 6 nearly all have some hydrogen bond acidity, but the values of $\Sigma\alpha_2^H$ are all much lower than expected for NH acids. The $\Sigma\alpha_2^H$ values for Z–Ala–Val–OMe (0.24) should be understood as containing the hydrogen bond properties of *N*-methylacetamide (0.4),

Table 7
Solute descriptors of tripeptide derivatives[a]

Peptide	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	π_2^H	R_2	$V_x/100$	S.D. (CHI units)
Z–Ala–**Tyr(OBzl)**–Val–OMe	−0.09	2.14	8.66	2.475	4.584	1.61
Z–Ala–**Trp**–Val–OMe	0.07	2.01	6.91	2.600	3.889	0.68
Z–Ala–**Lys(NBzl)**–Val–OMe	−0.02	1.47	2.66	1.773	2.489	3.09
Z–Ala–**Asp(OBzl)**–Val–OMe	0.07	2.04	7.05	1.863	4.117	0.24
Z–Ala–**Ser(OBzl)**–Val–OMe	0.09	1.99	6.59	1.779	3.976	0.38
Z–Ala–**Phe**–Val–OMe	0.09	1.96	6.12	1.807	3.776	0.71
Z–Ala–**Leu**–Val–OMe	0.22	1.88	5.28	1.096	3.591	1.34
Z–Ala–**Tyr**–Val–OMe	0.36	2.06	6.49	2.047	3.835	1.01
Z–Ala–**Val**–Val–OMe	0.25	1.87	5.05	1.091	3.450	1.40
Z–Ala–**His**–Val–OMe	0.56	2.15	5.53	1.884	3.596	1.91
Z–Ala–**Ser**–Val–OMe	0.48	1.96	4.51	1.339	3.227	1.33
Z–Ala–**Asn**–Val–OMe	0.54	2.10	4.96	1.439	3.425	1.42

[a] The order of the tripeptide derivatives was chosen according to their CHI value ranking (Table 5) including the tripeptide derivatives with protected polar side chains.

acetanilide (0.5) and methylacetate (0.4). The discrepancy between the sum of the substance contributions and the experimental data show the complexity of hydrogen bonding acidity and basicity for peptide derivatives, where conformational effects and intermolecular interaction have to be considered. For larger peptides and proteins the formation of tertiary structures becomes essential and thus we expect an even larger influence of the conformation on the hydrogen bond properties than for the oligopeptides tested in this work.

Similarly, all the peptides in Table 6 act as hydrogen bond bases due to the urethane, peptide and ester carbonyl groups. Although not as severe as found for the $\Sigma\alpha_2^H$ values the $\Sigma\beta_2^H$ values for the peptides are lower than expected. These results suggest that for the peptides in Table 6 values of $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ are not simply the sum of the individual values of smaller molecules containing the same acceptor or donor functions. Not only may one functional site in the molecule influence acidity and basicity of another site in the same molecule, but conformation in solution and intramolecular interaction may affect the total overall hydrogen bond acidity and basicity of the peptide.

3.2.2. Relationship between hydrophobicity and solute descriptors (Table 7)

The compounds in this Table were ordered according to their CHI values e.g., to the decreasing hydrophobicity. Thus, looking at the tendencies of $\Sigma\beta_2^H$ and π_2^H we found that both parameters generally decrease with decreasing hydrophobicity. In con-

Table 8
Influence of the chirality on solute descriptors of tripeptide derivatives

Peptide	$\Sigma\alpha_2^H$	$\Sigma\beta_2^H$	π_2^H	S.D.	K (l/mol)[a] [38]
Z–Ala–**Phe**–Val–OMe					
LLL	0.10	1.96	6.12	0.71	0.65
LDL	0.12	1.94	6.15	0.61	0.85
DLL	0.10	1.94	6.16	0.74	0.77
Z–Ala–**Leu**–Val–OMe					
LLL	0.22	1.88	5.28	1.34	0.63
LDL	0.19	1.87	5.26	0.99	0.95
DLL	0.22	1.88	5.28	1.20	–

[a] At 25°C.

trast the hydrogen bond acidity increases in the same order. Furthermore, protection of the polar functions in the side chain results in an increase of the hydrogen-bond basicity because by blocking the OH and NH functions, they are converted into stronger hydrogen bond bases. Deviations from the relation between hydrophobicity and $\Sigma\beta_2^H$ and π_2^H were observed in the case of the tripeptide with Tyr, Ser and Asn as the second amino acid residue. This can be easily understood from the structure of those amino acids. Tyr and Ser have polar OH function in the side chain. Thus, one would expect that this function works most probably as a proton donor which might compensate for the influence of the hydrogen bond basicity by a higher hydrogen bond acidity, and thus result in the ranking of this derivative in the hydrophobicity scale. Indeed also the hydrogen bond acidity of those tripeptides is surprisingly large. For *N*- and *C*-terminal protected peptide derivative we would have expected values close to zero as shown in [37].

In the case of the Tyr(OBzl) containing tripeptide derivative we observe a high hydrogen bond basicity which is not compensated by the hydrogen bond acidity. Thus, the hydrophobicity of this substance is also large. In the case of Asn as Xaa in the tripeptide derivative there is also another amide group in the side chain which results in a higher overall basicity which is also compensated by $\Sigma\alpha_2^H$.

3.2.3. Chirality of the tripeptide derivatives (Table 8)

Our calculation of V_x and R_2 is the same for different diastereoisomers. Thus, the differences in the CHI values must be interpreted as due to different hydrogen bond interactions between the peptide derivative and the stationary phase, or conformational effects of the peptide derivative itself. In the case of the hydrogen bond basicity it was clearly found that all LLL diastereoisomers show slightly greater values for $\Sigma\beta_2^H$. A former paper [38] discussing infrared data on the intramolecular association behaviour of these peptides had shown that the diastereoisomers of these tripeptide derivatives differ in their equilibrium constants of intramolecular association. However, due to the steric hindrance in these structures the LDL diastereoisomer exhibited the highest equilibrium constant whereas DLL and LLL form behaved similar. Our $\Sigma\alpha_2^H$ data do not allow such a clear differentiation. The dipolarity/polarisability parameter which reflects the conformational changes within the tripeptides does not show any significant effect.

4. Conclusions

Our investigations have shown that the fast-gradient HPLC leads to a new chromatographic hydrophobic index which can be used as a solute property in QSPR equations. For a number of tripeptide derivatives we have used this CHI value for the estimation of solute descriptors which represent structural differences in an accurate manner. It is even possible to distinguish diastereoisomeric tripeptide derivatives with respect to their different hydrogen bond basicity. This fast gradient method is thus a very fast method to determine detailed structural parameters of similar oligopeptide derivatives. On the hand of the five descriptors in Eq. (1) these molecules can be characterised in a comprehensive manner. Furthermore, based on those descriptors it is possible to explain the hydrophobicity of molecules in dependence of composition, sequence and chirality in terms of hydrogen bond basicity and acidity as well as dipolarity/polarisability. Thus, if we can understand the conformational and steric effects and their influence on the hydrophobicity we hope to receive a tool for the prediction of partition coefficients based on the structure of the peptide and proteins. We will continue to study the effects of sequence changes in more detail and complete our set of solute descriptors of peptide derivatives.

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