Organic & Biomolecular Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/obc

The effect of N-methylation of amino acids (Ac-X-OMe) on solubility and conformation: A DFT study

Shah Md. Abdur Rauf,[a] Per I. Arvidsson,[a,b] Fernando Albericio,[a,c,d,e,f,g] Thavendran Govender,[a] Glenn E. M. Maguire,[a,g] Hendrik G. Kruger[a,*] and Bahareh Honarparvar.[a,*]

[a]Catalysis and Peptide Research Unit, School of Health Sciences, University of KwaZulu-Natal, Durban 4001, South Africa.

[b]Science for Life Laboratory, Drug Discovery & Development Platform & Division of Translational Medicine and Chemical Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden.

[c]Institute for Research in Biomedicine-Barcelona, 08028-Barcelona, Spain.

[d]CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Barcelona Science Park, 08028-Barcelona, Spain.

[e]Department of Chemistry, University of Barcelona, 08028-Barcelona, Spain.

[f]School of Chemistry, Yachay Tech, Yachay City of Knowledge, 100119-Urcuqui, Ecuador.

[g]School of Chemistry and Physics, University of Kwazulu-Natal, Durban 4001, South Africa.

***Corresponding authors:** Honarparvar@ukzn.ac.za (Bahareh Honarparvar), kruger@ukzn.ac.za (Hendrik G. Kruger), Telephone: + 27 31 2601845, Fax: +27 31 2603091, Catalysis and Peptide Research Unit, School of Health Sciences, University of KwaZulu-Natal, Durban 4041, South Africa.

Abstract

N-Methylation has a significant impact on improving oral bioavailability, lipophilicity and aqueous solubility of peptide-based lead drug structures. The selected mono-amino acid derivatives Ac-X-OMe, where X= Gly, Val, Leu, Ile, Phe, Met, Cys, Ser, Asp and His as well as their corresponding N-methylated analogues were studied. The Clog*P* values of all N-methylated examples are greater than native compounds. Quantum chemical calculations were performed to estimate the aqueous solubility of these lipophilic compounds using density functional theory (DFT) with B3LYP density functional. To confirm the contribution of dispersion forces on quantum chemical data, the long-range corrected (LC) hybrid density functional (ωB97X-D)

was also probed for some amino acid derivatives. The ωB97X functional gave similar results. Our results reveal that after mono N-methylation of the peptide backbone, ΔG_{solv} becomes more negative (more water soluble) while polarizability, dipole moment are also increased. NBO atomic charges for the amide N, C and O nuclei become more positive/(less negative) after N-methylation. All N-methylated amino acids have higher E_{HOMO} (less negative) in comparison to the amino acid analogues, and in all cases N-methylation decreases $E_{HOMO-LUMO}$. The calculated amide *cis/trans* activation energy (E_A) of all the N-methylated amino acid derivatives was lower than native species. N-methylation of these compounds leads to an increase in lipophilicity, aqueous solubility, polarization, dipole moment and lowering of the *cis/trans* amide energy barrier (E_A).

Keywords: N-methylation • Peptides • Density functional theory (DFT) • Natural bond orbital (NBO) analysis • Bioavailability • Solubility • Lipophilicity.

Introduction

Biologically active compounds are not always good drug candidates; one reason for this is low bioavailability after oral administration. Drug candidates with reasonable absorption, stability, and low cellular toxicity, have a higher probability of progression through the drug discovery and development pathway. In this context, proteins and peptides are promising scaffolds for predictable and selective biological response;[1] moreover several methods are available for analyzing their mode of actions.[2-5] However, many peptides have low bioavailability due to proteolysis and poor membrane permeability,[6] which limit their application as drugs.[7, 8] In view of this, medicinal chemists have developed an array of strategies to solve these problems, including incorporation of peptide bond isosters, peptoids, retro-inverso peptides, structural modifications such as covalent attachment of poly-ethylene glycol (PEG), lipidation and other chemical modifications.[9-12]

N-Methylation of the amide bond is an effective technique to increase the proteolytic stability[13-17] of peptides, and to improve pharmacological properties, particularly bioavailability[16, 18-22] and lipophilicity.[14, 23, 24] N-Methylation scanning is an approach by which a library of all possible N-methylated peptide analogues, are synthesized and screened to select the most promising N-methylated lead peptide.[19, 25, 26] N-Methylation of peptides also improves the ADMET properties of such compounds.[18, 23] It has been reported that incorporation of N-methyl amino acids enhances the potency and the receptor subtype selectivity of peptides.[17, 27-

32] However, exceptions to these experimental observations have been reported.[27, 33, 34] Peptides containing single or multiple N-methylations are promising agents to disrupt protein–protein hydrogen bond interactions involving β-sheet interactions, as illustrated by inhibitors for amyloid β-peptide (Aβ)[35-38] and amylin[39-44] fibrillation.

Tonelli demonstrated that N-methylated tripeptides,[45] as well as N-methylated gramicidin,[46] experience conformational rigidity due to specific hydrogen bonding interactions, despite increased *cis*-amide conformations. Several computational[30, 32, 47-53] and experimental[17, 30, 48, 50, 51, 54-59] reports on other peptides reports support these experimental observations.

Recently our NMR and molecular modelling results revealed that N-methylation of amide/peptide bonds containing proline N-oxides in short acyclic peptides lead to an increase of the *cis/trans* ratio but simultaneous induces specific hydrogen bond interaction that stabilize certain conformations (such as quasi β-turns).[60] N-Methylation of short cyclic peptides also revealed more rigid behaviour according to NMR solution and molecular dynamics studies.[51, 61]

Motivation for our study came from the experimental observation by Bose *et al.*[23, 38] where it was reported that N-methylation simultaneously enhanced both the lipophilicity and aqueous solubility of short segments of the Aβ peptide that were used as aggregation inhibitors for Alzheimer's disease. They reported that the solubility increased ~50 - 1000 fold through N-methylation in the case of linear hexapeptides (1 – 5 methyl groups). However, for short cyclic peptides, N-methylation was reported to cause a decrease in aqueous solubility.[24] This was ascribed to the increase in lipophilicity.

It is clear from literature that an improved understanding of the effect of N-methylation on aspects such as solubility (N-methylated peptides are both more water soluble and more lipohilic) and flexibility (such peptides are more flexible in terms of *cis/trans* interconversion, but simultaneously also more rigid) is required. We attempted a theoretical study to shine light on these dual characteristics.

Physicochemical and quantum-chemical descriptors[62-64] including Partition coefficient (Clog*P*),[65-69] Gibbs free solvation energy (ΔG_{solv}), polarizability (α), dipole moment (μ) and $\Delta E_{HOMO\text{-}LUMO}$, which are related to aqueous solubility[70-72] are used as modern predictive models for drug absorption.[73] Solubility is also a fundamental feature of drug absorption; a drug must be reasonably soluble in an aqueous environment for subsequent suitable ADMET characteristics.[74]

In this study, a detailed theoretical investigation was performed to address the impact of N-methylation of the amide bond on particularly lipophilicity, aqueous solubility and reactivity of

the selected non-polar aliphatic/branched chain, polar charged/uncharged, and aromatic L-amino acid derivatives as Ac-X-OMe where X= Gly, Val, Leu, Ile, Phe, Met, Cys, Ser, Asp and His and their N-methylated analogues (**Figure 1**). Furthermore, a conformational analysis was also carried out to assess the effect of N-methylation on the energy barrier (E_A) for *cis/trans* conformational changes.

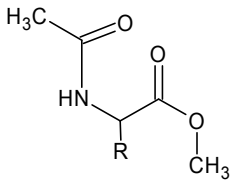
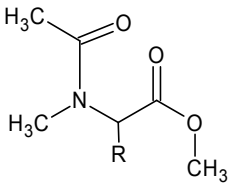
Amino acid derivatives Ac-X-OMe	**Amino acid derivative (native form)**	**N-Methylated Amino acid derivative**
X= Gly, Val, Leu, Ile, Phe, Met, Cys, Ser, Asp and His		

Figure 1. The general structure of the selected L-amino acid derivatives, Ac-X-OMe and their corresponding N-methylated analogues.

Results and discussion

Solubility in terms of drug bioavailability requires knowledge about the lipophilic (ability to cross membranes) and the hydrophilic (solubility in blood plasma) nature of the compound.[75-77] Even though enough experimental results are available for a general correlation of the theoretical results, not enough experimental results are available to perform a statistical analysis to determine the accuracy of that. In absence of such data, we have looked at similar results from literature[78-84] that suggest the accuracy of the calculations warrants one or two decimal places.

Lipophilicity

High values of log*P* is an indication of a large lipophilic character for a compound.[65] The calculated Clog*P* of the selected amino acid derivatives, Ac-X-OMe (X= Gly, Asp, Ser, Val, Leu, Ile, Cys, Met, His and Phe) and their corresponding N-methylated analogues are shown in **Table 1**. All the N-methylated compounds exhibit considerable increases in the calculated Clog*P* values, in comparison to the corresponding native amino acid derivatives.

Table 1. Calculated Clog*P* of the selected amino acid derivatives, Ac-X-OMe (X= Gly, Asp, Ser, Val, Leu, Ile, Cys, Met, His and Phe) and their corresponding N-methylated analogues.

Ac-X-OMe	Native	N-Methylated
X= Gly	-1.07	-0.31
X= Val	0.16	0.92
X= Leu	0.16	0.98
X=Ile	0.69	1.45
X= Phe	2.03	2.57
X= Met	0.15	1.09
X= Cys	0.17	0.93
X= Ser	-1.43	-0.41
X= Asp	0.47	0.96
X= His	-1.30	-0.52

The range of increment in Clog*P* is between 0.49 and 1.02. In general, the Clog*P* values increase more in hydrophobic/nonpolar amino-acids than polar amino acids. Among the non-polar amino acids, Phe has the smallest Clog*P* increment upon N-methylation. On the other hand, in polar amino acids the change is smaller except for Ser. Among polar amino acids, Asp also has the smallest Clog*P* increment.

Aqueous solubility parameters

The Gibbs free solvation energy (ΔG_{solv}), polarizability (α), dipole moment (μ), and frontier molecular orbital energies (E_{HOMO}, $E_{HOMO\text{-}LUMO}$) of the selected models were also calculated (B3LYP/6-311++G**) and the results are reported in **Table 2**. The aqueous solubility of the considered molecules was assessed by measurement of their Gibbs free solvation energy (ΔG_{solv}) using the polarizable continuum model (PCM). Comparison of the ΔG_{solv} data revealed higher solubility for all the N-methylated models (with more negative ΔG_{solv} values) (**Table 2**). Solubility is relatively higher (less negative ΔG_{solv}) in polar amino acids than that of non-polar amino acids. Among the polar amino acids, His exhibits the highest aqueous solubility. Among the non-polar amino acids Phe has the highest solubility (less negative ΔG_{solv}). These data correlates with other descriptors like polarizability and dipole moments.

Polarizability

The polarizability is defined as the linear coefficient between an applied electric field and the induced dipole moment. Molecular polarizability of a molecule reflects the global polarity of the molecular structure[85] that results from the uneven partial charge distribution over all the atoms of the molecule. Normally, highly polar compounds with reasonable inter-molecular electrostatic interactions are more water soluble.[86, 87] As shown in **Table 2**, the computed polarizabilities increased for all amino acid derivatives Ac-X-OMe after N-methylation both in gas phase and in

water. The higher values of polarizability for the N-methylated cases are more evident in water medium in comparison to the gas phase.

Table 2. ΔG_{solv} (kcal/mol), polarizability (α, Å^3), dipole moment (μ, Debye) and frontier molecular orbital energies (E_{HOMO}, $E_{HOMO\text{-}LUMO}$, eV) of the selected amino acid derivatives, Ac-X-OMe* and their corresponding N-methylated analogues with different density functionals.

Ac-X-OMe	B3LYP/6-311++G(d,p)						
	ΔG_{solv} (kcal/mol)	α (A^3)		μ (Debye)		E_{HOMO} (eV)	$E_{HOMO\text{-}LUMO}$ (eV)
		Gas	Water	Gas	Water	Gas	Water
X=Gly	-7.530	37.751	49.208	3.215	4.472	-6.860	6.606
X=(NMe)Gly	-8.157	43.525	58.008	3.498	5.481	-6.582	6.525
X= Val	-7.731	55.468	75.577	4.537	5.774	-7.071	6.653
X=(NMe)Val	-7.798	61.517	84.806	4.999	6.718	-6.618	6.508
X= Leu	-6.876	62.305	84.922	2.957	3.797	-6.858	6.600
X=(NMe)Leu	-7.606	68.190	89.939	3.796	4.860	-6.613	6.424
X= Ile	-7.017	61.638	84.277	2.718	3.507	-6.595	6.388
X=(NMe)Ile	-7.770	67.708	93.437	2.988	3.990	-6.422	6.246
X= Phe	-8.812	75.513	105.513	2.952	3.759	-6.112	5.723
X=(NMe)Phe	-10.092	81.694	114.959	3.353	4.257	-5.813	5.366
X= Met	-8.597	65.067	87.589	4.619	6.014	-5.878	5.389
X=(NMe)Met	-9.202	71.264	96.691	4.836	6.277	-5.781	5.222
X= Cys	-7.412	52.478	69.962	4.426	5.465	-6.539	6.051
X=(NMe)Cys	-9.184	58.659	79.461	4.751	6.098	-6.435	5.796
X= Ser	-9.126	45.731	60.612	4.080	5.552	-6.610	6.265
X=(NMe)Ser	-9.612	51.473	69.211	4.952	6.238	-6.574	5.925
X= Asp	-11.617	51.695	52.735	7.351	8.856	-7.239	6.584
X=(NMe)Asp	-12.968	57.437	68.788	9.630	11.890	-6.936	5.919
X= His	-12.699	68.904	94.204	9.054	9.211	-5.731	4.890
X=(NMe)His	-13.419	74.677	103.063	9.504	12.004	-5.651	4.625
ωB97XD /6-311++G(d,p)							
X= His	-16.801	63.951	85.743	7.682	10.571	-7.936	6.399
X=(NMe)His	-17.858	69.703	94.548	8.826	11.714	-7.873	6.395

- Cartesian coordinates of the optimized structures are presented with the supplementary material.

-740.514152

Histidine exhibited the largest polarizability values among the polar amino acids and Phe gave the largest polarizability values among the non-polar ones, while Gly (a small and non-polar amino acid) had the smallest polar character. In general, more polarized compounds are expected to be more soluble in water.[88] The significant increase in polarizability upon N-methylation will modify the interaction of amino acid derivatives with polar solvents such as water and ultimately increase their solubility in biological systems including body fluids.[87]. The same trend for the N-methylation effect was observed with the ωB97XD density functional for His/(NMe)His. This functional incorporates dispersion effects.

Dipole moment

Dipole moment is the product of all the charges on a molecule and the distance of separation between them. There is also a direct relationship between polarizability and dipole moment.[89-91] Many polar drug-like molecules form dipole moments resulting from intra-molecular charge transfer. The size of the molecular dipole moment influences the solubility of the compound in various solvents. **Table 2** presents the important increase of the dipole moments of the N-methylated derivatives, compared to their corresponding native amino acids. In all 10 examples, the dipole moments increased both in the gas and liquid phases. The substantial increase in these values indicates an intensifying of the interactions of these derivatives with polar solvents such as water, and will thus enhance their solubility in physiological media.

In general, ΔG_{solv} values, polarizibility, and dipole moments are smaller in hydrophobic amino acid derivatives than for the polar/hydrophilic series. Among the hydrophobic amino acid derivatives Gly has the smallest quantum chemical descriptors (ΔG_{solv}, polarizability and dipole moment) which are ascribed to a lack of side chain (R = H). The values of these quantum chemical descriptors increase with the elongation of aliphatic side chain with Leu and Ile having similar properties. Among the non-polar amino acid categories Phe, with an aromatic side chain, has the highest descriptor values. In the series of the polar amino acid derivatives, Ser and Cys with short uncharged side chain exhibit smaller descriptor values. Asp and His exhibit higher descriptor values due to charged side chains.

Analysis of atomic charges

Solubility, molecular polarizabilities and dipole moments are fundamentally linked to individual atomic charges. Natural bond orbitals (NBO) analysis is an efficient method for studying intra and inter-molecular bonding and interactions among bonds, which also provides a convenient basis for investigation of charge transfer or conjugative interactions in molecular systems.**[92]**

The natural atomic charges (nuclear charge minus summed natural populations of NAOs on the atom and total core, valence, and Rydberg populations on each atom) derived from NBO analysis of two selected amino acid derivatives, Ac-X-OMe (X= Gly and Val) and their corresponding N-methylated models are demonstrated in **Figure 2.**

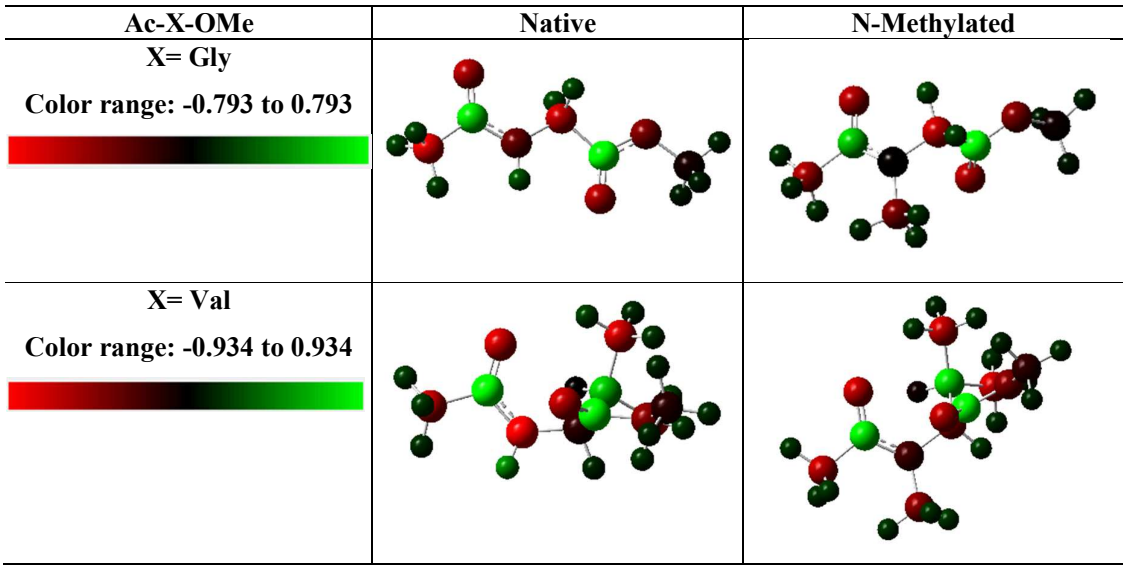


Figure 2. Natural atomic charge derived from natural bond orbital analysis of two amino acid derivatives, Ac-X-OMe (X= Gly and Val) and their corresponding N-methylated analogues obtained using B3LYP/6-311++G(d,p). The nitrogen atom of the amide bond, appeared less negative for the N-methylated cases. This fact is clearly evident from the value's range presented by its corresponding colour code. The natural atomic charges of the rest of the compounds are provided in supplementary information. The results for the other structures are provided in supplementary information)

The obvious change in charge is that of the amide nitrogen, which becomes more positive upon N-methylation. This is due to the increased double bond character for the amide CN bond, yielding a partial positive charge on the amide nitrogen.

Figures 2, 3 (See also **Table 1S**, Supplementary material – charges for each atom presented) show the change of the natural atom charges due to N-methylation. The changing trend of each individual atomic charge is difficult to follow, so we investigated the variation of a number of functional group charges. Functional groups were defined as demonstrated in **Figure 3** and the sum of the atom charges of each group are presented in **Table 3**.

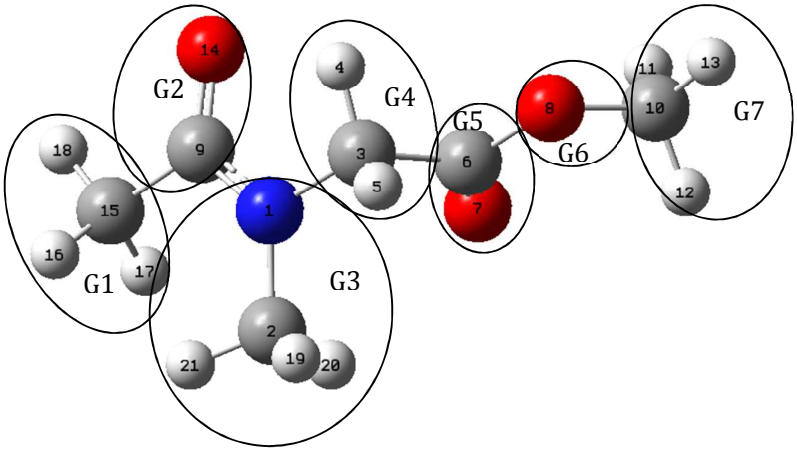


Figure 3. The labelled structure of Ac-X-OMe (X= Gly) with the representative functional groups.

Table 3. The effect of N-methylation through the inductive effect of the methyl group on the atomic charges (both NBO and ESP) distributed on Ac-X-OMe (X= Gly) calculated with different density functionals. Each backbone atom, along with the H or O considered as a group. Sum of charges of atoms in a group considered as a group charge.

Group (G)	B3LYP/6-311++G(d,p) Natural atomic charges (a.u.)	
	Ac-Gly-OMe	Ac-(NMe)Gly-OMe
G_1 (CH_3)	0.004	0.006
G_2(CO)	0.025	0.052
G_3(NMe)	-0.229	-0.265
G_4(CH_2)	0.195	0.221
G_5(CO)	0.194	0.211
G_6(O)	-0.547	-0.559
G_7(CH_3)	0.359	0.352
	ωB97XD /6-311++G(d,p)	
G_1 (CH_3)	-0.018	-0.015
G_2(CO)	0.066	0.089
G_3(NMe)	-0.239	-0.270
G_4(CH_2)	0.168	0.181
G_5(CO)	0.221	0.227
G_6(O)	-0.540	-0.549
G_7(CH_3)	0.342	0.338

These results show that the inductive effect of N-methyl in G_3 causes the functional group NBO charge to become more negative. As a result, the charges of the neighbouring groups – G_2 and G_4 become more positive. Similar induced functional group charges are also evident for G_5 and G_6. The inductive effects on atomic charges are similar for both B3LYP/6-311++G(d,p) and ωB97XD /6-311++G(d,p) functionals, except for G_1 that became negative. This effect causes polarization through charge transmission, which ultimately appears to increase the solubility of peptides in aqueous solution. Tajkhorshid and Suhai reported similar results in a theoretical investigation of a N-methylated conjugated alkene system.[93]

Frontier molecular orbitals (HOMO, LUMO)

Molecular polarizability and atomic electron densities/charges are directly linked to the frontier molecular orbitals.[94, 95] The latter is therefore also linked to solubility.[96-99] E_{HOMO} describes the tendency of the molecule to donate electrons (charge density). In general: the higher the E_{HOMO} energy the greater the ability of the molecule to donate electrons. The data in **Table 1** shows that all N-methylated structures have higher E_{HOMO} (less negative) in comparison to their native amino acids. The energy difference between the HOMO and LUMO frontier orbitals ($E_{HOMO-LUMO}$ gap) is an important characteristic of chemical reactivity.[100, 101] Large $E_{HOMO-LUMO}$ energy differences indicate that the available electrons in these molecules have less tendency to move to the excited state and such compounds are chemically more inert.[87] **Table 2** reveals a decrease in $E_{HOMO-LUMO}$ after N-methylation, which shows the more polarized nature[102-104] of these compounds. In terms of solvent effects, it suggests that N-methylated peptides interact more with polar solvent molecules and repel non-polar solvent molecules. In terms of amide *cis-trans* interchange, smaller $E_{HOMO-LUMO}$ differences also suggest more facile excitation to the triplet state, which is required for rotation around the N-C bond.[105, 106]
Specific grid points are sampled for HOMO and LUMO frontier orbitals and a viewable surface is formed[107] which is demonstrated in **Figure 4**. Inspection of the HOMO orbitals of these compounds reveals that the charge density is predominantly located at the amide bonds.

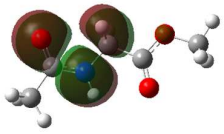
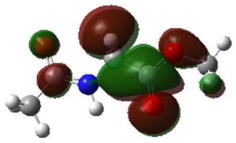
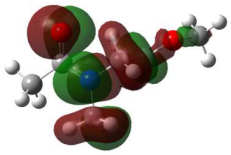
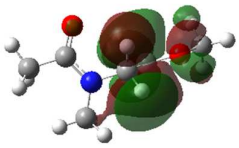
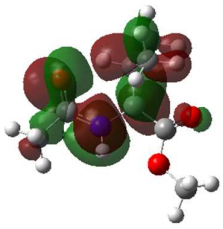
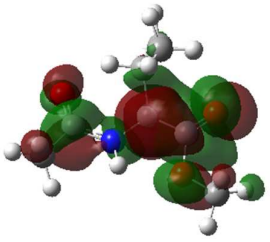
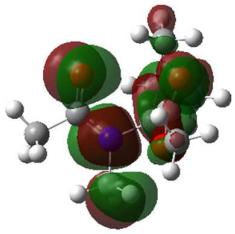
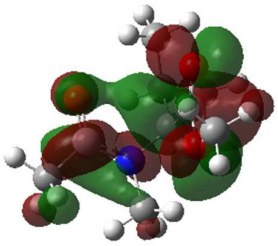
Amino acid derivatives Ac-X-OMe	HOMO	LUMO
X= Gly		
X= (NMe)Gly		
X=(NMe)Val		
X=(NMe)Val		

Figure 4. The frontiers orbitals (HOMO and LUMO) of two amino acid derivatives, Ac-X-OMe (X= Gly and Val) and their corresponding N-methylated models obtained using B3LYP/6-311++G(d,p). (HOMO and LUMO representations of the rest of considered compounds are provided in supplementary information).

The calculated $E_{HOMO\text{-}LUMO}$ values for the *cis/trans* conformation and TS for Ac-Gly-OMe its corresponding N-methylated analogues in vacuum, water and cyclohexane media are reported in **Table 4**.

Table 4. The calculated $E_{HOMO-LUMO}$ values for the *cis/trans* conformation and TS for Ac-Gly-OMe and its corresponding N-methylated analogues in gas, water and cyclohexene media using different density functionals.

Ac-Gly-OMe	$E_{HOMO-LUMO}$ (eV) Gas		$E_{HOMO-LUMO}$ (eV) Water		$E_{HOMO-LUMO}$ (eV) Cyclohexane	
	Native	N-Met	Native	N-Met	Native	N-Met
trans	6.593	6.588	6.997	6.810	6.763	6.694
TS	6.012	5.463	6.066	5.493	6.029	5.475
cis	6.417	6.458	6.917	6.739	6.647	6.567
ωB97XD /6-311++G(d,p)						
trans	6.468	6.452	6.794	6.635	6.547	6.330
TS	6.001	5.702	6.018	5.269	6.001	5.331
cis	6.398	6.371	6.857	6.638	6.592	6.460

The obtained data in **Table 4** implies that for most cases (except the *cis*-form in the gas phase) the $E_{HOMO-LUMO}$ band gap is reduced after N-methylation in all media. These results indicate that N-methylation facilitates the *cis/trans* inter-conversion. The band gaps for the N-methyl transition states in all media are the smallest. This suggests that the N-methylated transition states are most likely to be excited to the triplet state, which is required for rotation around the amide C-N bond.[105, 106]

It became clear that classical resonance structures[108, 109] proposed to describe the nature of the amide bond is not satisfactory.[110-116] It does not account for the fact that upon rotation of the amide bond, the C-O bond length decreases very little (0.01 Å), while the C-N bond lengthens much more (0.08 Å).[115] Second-order perturbation analysis (to determine stabilization through hyperconjugation)[117] of the natural bonding orbitals was used to show that, although C-N rotation disrupts the amide resonance energy [n(N)→π*(CO)], it introduces a smaller hyperconjugative stabilizing interaction [n(N)→σ*(CO)] of 9.6 kcal/mol. This explains the difference between vertical resonance stabilization energy values (25 kcal/mol) and rotation barriers (15 kcal/mol) for amides.[115] The same trend for the N-methylation effect was observed for $E_{HOMO-LUMO}$ values for the *cis/trans* conformation and TS for Ac-Gly-OMe and its corresponding N-methylated analogues in gas, water and cyclohexene media using the ωB97XD density functional.

Electron delocalization between occupied Lewis-type (bond or lone pair) NBO and unoccupied non-Lewis NBO orbitals corresponds to a stabilizing donor-acceptor interaction. This can be calculated with second-order perturbation calculations, providing information about hyperconjugation. We therefore investigated the effect of N-methylation on hyperconjugation for three cases: *cis*- and *trans*-glycine as well as the transition state. The two lone pairs on the amide oxygen and the lone pair on the amide nitrogen can potentially play a role in hyperconjugation

around the amide bond. The calculated results are presented in **Table 5**.

Table 5. Second-order perturbation theory analysis of the Fock matrix[118] in NBO basis corresponding to the intra-molecular bonds of native and N-methylated analogue of *cis/trans* Ac-Gly-OMe and its corresponding N-methylated analogues in gas phase, water and cyclohexane media using different density functionals.

Amide bond		E(2)/kcal mol^{-1}					
		B3LYP/6-311++G(d,p)					
Donor	Acceptor	Gly *trans* - Gas		Gly TS - Gas		Gly *cis*- Gas	
		Native	N-met	Native	N-met	Native	N-met
LP1 O_{14}	σ*N_1-Me	24.31	24.41	28.01	28.15	24.73	25.25
LP2 O_{14}	σ*N_1-Me	1.63	1.71	1.53	1.67	1.66	1.75
LP N_1	σ*C_9-O_{14}	63.67	60.02	9.48	9.19	60.89	57.46
Donor	Acceptor	Gly *trans* - Water		Gly TS - Water		Gly *cis* - Water	
		Native	N-met	Native	N-met	Native	N-met
LP1 O_{14}	σ*N_1-Me	23.79	23.89	27.68	27.75	24.13	24.68
LP_2 O_{14}	σ*N_1-Me	1.57	1.66	1.49	1.65	1.60	1.70
LP N_1	σ*C_9-O_{14}	67.72	64.11	9.52	9.26	65.21	61.48
Donor	Acceptor	Gly *trans* - Cyclohexane		Gly TS - Cyclohexane		Gly *cis* - Cyclohexane	
		Native	N-met	Native	N-met	Native	N-met
LP_1 O_{14}	σ*N_1-Me	24.09	24.21	27.87	28.01	24.49	25.03
LP_2 O_{14}	σ*N_1-Me	1.61	1.69	1.51	1.66	1.63	1.73
LP N_1	σ*C_9-O_{14}	65.21	61.50	9.50	9.21	62.63	58.93
ωB97XD /6-311++G(d,p)							
Donor	Acceptor	Gly *trans* - Cyclohexane		Gly TS - Cyclohexane		Gly *cis*- Cyclohexane	
		Native	N-met	Native	N-met	Native	N-met
LP_1 O_{14}	σ*N_1-Me	30.60	29.87	38.72	30.60	38.70	39.96
LP_2 O_{14}	σ*N_1-Me	0.82	1.67	0.55	1.67	1.08	1.71
LP N_1	σ*C_9-O_{14}	92.84	83.40	10.27	8.93	88.92	83.65

In second-order perturbation analysis of NBOs, larger E(2) values represent stronger interaction between electron-donors and electron-acceptors in a molecule (increased hyperconjugative interaction of the system).[119-122] The largest contribution to stabilization of the amide bond through hyperconjugation is indeed due to [n(N)→σ*(CO)]. Interestingly, after N-methylation, this stabilization effect for the amide bond is smaller in all cases, which is indicative of reduced double bond character (N-methyl peptides have slightly longer C-N bonds – see Table 2S, Supplementary material). This hyperconjugative interaction is optimal for the planar amide bonds and is abridged upon rotation as is evident for the transition state.

Stabilization as result of [n(O)→σ*(NC)] hyperconjugation (about 24 – 28 kcal mol^{-1}) is smaller than that of [n(N)→σ*(CO)] for the planar conformations (about 58 – 65 kcal mol^{-1}) but larger for the corresponding transition states (about 28 kcal mol^{-1} for [n(O)→σ*(NC)] and 9 kcal mol^{-1}

for [n(N)→σ*(CO)]). In all cases after N-methylation, one lone pair of O_{14} contributes to stronger hyperconjugative stabilization (about 28 kcal mol^{-1}) through interaction with N_1-C_9 antibonding sigma bonding orbitals than the native states (about 24 kcal mol^{-1}). This further supports the experimental observation that the energy barrier for *cis-trans* interconversion of N-methylated amides is lowered. The same trend for the N-methylation effect was observed for electron delocalization for the *cis/trans* and TS of Ac-Gly-OMe and its corresponding N-methylated analogues in cyclohexene medium using ωB97XD density functional. In general smaller differences were obtained with ωB97XD.

Conformational study

The effect of N-methylation of amino acids on the conformational change (rotation of amide bond) of such compounds was also investigated. We postulated that such knowledge will also shine light on the difference in aqueous solubility of short acyclic (increased solubility)[23] *versus* cyclic (decreased solubility)[24] N-methylated peptides.

The energy of activation (E_A) between the amide *cis/trans* conformations were determined by calculating the transition state (TS) for the selected amino acid derivatives, Ac-X-OMe (X= Gly, Asp, Ser, Val, Leu, Ile, Cys, Met, His and Phe) and their corresponding N-methylated analogues. These are presented in **Table 6**. Note that for each case, two possible transition states exist, one where the backbone dihedral angle ($C_α$-CO-NH-CO) is at about 60 degrees (TS_1) and the second transition state at about 100 degrees (TS_2).

The results reveal that in all the considered short peptides, the E_A for both cases (TS1 and TS2) were significantly decreased after N-methylation. This is in agreement with an *ab initio* investigations by Bagayi *et al.*[49] Despite an isolated claim to the contrary,[14] it is known from experimental studies that the *cis/trans* interconversion around the amide bond is facilitated through N-methylation.[54, 123-126]

Table 6. Calculated energies and torsion angles for the conversion of *cis/trans*-amino acids (Ac-X-OMe) and their corresponding N-methylated analogues using different density functionals (the entropy contributions are provided with the supplementary material – Table 4S).

	TS1				TS2		
	B3LYP/6-311++G(d,p)						
Ac-X-OME	**$\Delta H_{cis\text{-}trans}$ /Kcal mol^{-1}**	**$E_{Acis/trans}$ /Kcal mol^{-1}**	**$E_{Atrans/cis}$ /Kcal mol^{-1}**	**Torsion angle/°**	**$E_{Acis/trans}$ /Kcal mol^{-1}**	**$E_{Atrans/cis}$ /Kcal mol^{-1}**	**Torsion angle/°**
=Gly	3.77	15.06	18.83	-54	18.83	22.59	108
=(NMe)Gly	1.26	17.57	18.20	-65	20.71	21.96	97
=Val	10.67	11.92	15.67	-64	19.45	30.12	97
=(NMe)Val	1.26	13.18	14.43	-49	18.20	19.45	76
=Leu	4.39	17.57	18.20	-51	21.96	21.96	110
=(NMe)Leu	0.63	15.69	16.32	-58	19.45	20.08	103
=Ile	10.67	12.55	15.69	-64	20.08	30.75	98
=(NMe)Ile	1.26	13.18	14.43	-49	18.20	19.45	77
=Phe	3.77	13.81	16.32	-68	19.45	23.22	111
=(NMe)Phe	1.88	14.43	15.68	-71	18.19	20.08	91
=Met	4.39	13.18	16.94	-50	19.45	23.84	111
=(NMe)Met	0.03	12.55	18.82	-68	18.82	25.10	93
=Cys	5.02	13.80	17.57	-67	18.20	23.21	95
=(NMe)Cys	3.14	15.06	16.94	-68	18.83	21.96	93
=Ser	4.39	13.81	18.20	-62	16.94	21.34	117
=(NMe)Ser	0.62	18.83	19.45	-71	18.20	18.83	90
=Asp	3.76	14.43	18.19	-64	19.45	23.22	115
=(NMe)Asp	1.26	16.94	18.19	-82	20.71	21.96	97
=His	6.28	14.43	20.71	-48	21.96	28.24	96
=(NMe)His	0.63	18.83	19.45	-85	21.34	21.96	95
ωB97XD /6-311++G(d,p)							
=His	4.39	15.68	20.08	-65	21.33	25.10	96
=(NMe)His	0.00	19.45	19.45	-67	19.45	19.45	94

$\Delta H_{cis/trans}$ = Energy difference between the *cis*-trans conformations.
$E_{Acis/trans}$ = Activation energy for the conversion of the *cis*-conformation *via* the transition state to the *trans*-conformation.
$E_{Atrans/cis}$ = Activation energy for the conversion of the *trans*-conformation *via* the transition state to the *cis*-conformation.
Torsion angle of the amide bond (C_α-CO-NH-CO)

Our calculations confirm that the *cis/trans* inter-conversion of the amide/peptide bond has a lower energy barrier (E_A) upon N-methylation and our results are in agreement experimental values.[55, 124]

A polar solvent, such as DMSO with a large dipolar moment (3.96 D), favours *cis*-isomers, while less polar solvents, such as water (1.85 D), induce more *trans*-character.[127, 128] Calculation of the energy barriers for Ac-X-OMe (X = Gly and Phe) in different solvents did reveal a significant difference between the native and N-methylated cases (Table 3S – supplementary material). For all planar cases (except for Ac-(NMe)Gly-OMe in water and cyclohexane where *cis* and *trans* have the same energies) the *trans* form has lower energies. The same trend of N-methylation effect on the energetic parameters for the *cis/trans* and TS of Ac-Gly-OMe and its corresponding

N-methylated analogues in gas phase using ωB97XD density functional for His/(NMe)His. Table 4S presents the calculated entropic contribution (ΔS) to the Gibbs free energy for the conversion of *cis/trans*-amino acids (Ac-X-OMe) and their corresponding N-methylated analogues. In all cases $\Delta S_{cis/trans}$ were considerably smaller for the N-methylated peptides. This suggests that N-methylation facilitates conformational interchange.

We have recently demonstrated (experimentally) that selective N-methylation of short proline N-oxide peptides induces increased *cis/trans* flexibility (depending on the position of N-methylation 29, 40, 50 and 77% *cis*-conformations were observed), facilitating quasi *β*-turn conformations.[60] Similar computational[30, 32, 47-53] and other experimental[17, 30, 47, 48, 50, 51, 54, 56-59] results have been reported, showing that N-methylation of the amide bond increase the *cis/trans* ratio. The fact that peptides become more flexible upon N-methylation will assist with solubility of acyclic peptides. Energy barriers between 15 and 20 kcal mol^{-1} can be overcome at room temperature and the energy barrier of amide rotation (from *cis* to *trans*) for N-methylated peptides is slightly lower than that.[55, 73, 92, 129] The calculated energy barriers for N-methylated peptides are in this range (Table 4), corresponding to experimental results that N-methylated peptides interconvert at room temperature between the *cis* and *trans* forms.

Peptides and proteins act in a certain sense like chameleons since in polar media the lipophilic parts are "hidden" from the surface of the protein, while the hydrophilic parts are exposed to the surface/water.[130, 131] On the other hand, when the protein enters a membrane, the protein rearranges so that the lipophilic parts are exposed to the surface. The increased *cis-trans* flexible nature of N-methylated peptides at room temperature facilitates this rearrangement of the peptide to optimize/minimize the interaction of polar/non-polar parts with the solvent. When this is combined with increased polarizability and dipole moment, the aqueous solubility of N-methylated peptides should be further increased. It also explains why short cyclic N-methylated peptides exhibit decreased water solubility since their *cis/trans* flexible nature is severely compromised and hydrogen bong interaction is also neutralised.[23, 38, 44] The authors have used NMR and computational techniques to argue that N-methylation of the compound destroys intramolecular hydrogen bonding in favour of intramolecular hydrogen bond interactions.[24] The decrease in aqueous solubility was ascribed to the increase in lipophilic character. Since the equivalent acyclic N-methylated peptides (with the same lipophilic nature) will be more water soluble, our explanation provides a more feasible rational for the loss of aqueous solubility.

Computational methods,[49, 52] especially molecular mechanics and molecular dynamics[30, 32, 47, 48, 50, 51, 53] are not compensating for the smaller force constant of N-methylated amide C-

N bonds, with the consequence that literature results portray a skewed picture of artificial increased rigidity for N-methylated peptides by not reflecting the increased *cis/trans* interconversion at room temperature.

Conclusion

The electronic structure properties of the selected amino acid derivatives, Ac-X-OMe (X= Gly, Asp, Ser, Val, Leu, Ile, Cys, Met, His and Phe) and their corresponding N-methylated analogues were investigated with B3LYP/6-311++G**. The effect of N-methylation on properties such as lipophilicity and solubility were evaluated. More negative ΔG_{solv} values higher values of polarizability and dipole moments for N-methylated amino acids in comparison to native amino acid series predict increased water solubility for the N-methylated compounds. In other words, the significant increase in polarizability of the N-methylated compounds is expected to modify the interaction of amino acid derivatives with polar solvents such as water, and consequently increase their solubility in the physiological medium, this is in agreement with previous experimental observations. The natural atomic charge of the amide bond N, C and O atoms become more positive upon N-methylation.

All N-methylated amino acids have a higher E_{HOMO} values than the amino acid analogues. Lower values of $E_{HOMO-LUMO}$ gap for N-methylated cases imply increased polarizibility and also increased possibility to be excited to the triplet state, especially for the transitions states (that exhibit the lowest $E_{HOMO-LUMO}$ gaps). The E_A values for *cis/trans* interconversion are considerably decreased after N-methylation, corresponding with experimental evidence that *cis/trans* interconversion occurs at room temperature.

The loss of hydrogen bond sites due to N-methylation is compensated for by the increased *cis/trans* flexibility of the peptide. Combination of this with increased polarizibility and dipole moment ensure suitable conformational changes to optimize/minimize the interaction of polar/non-polar parts with the solvent. Molecular mechanics/dynamics methods should introduce a more suitable force constant for N-methylated N-C amide/peptide bonds in order to accurately mimic N-methylated peptide folding.

Materials and methods

As the index of solubility and lipophilicity, Clog*P* values were estimated using ACD lab/Chemsketch[132]. All quantum chemical calculations were performed using Gaussian09 package[133] and Density Functional Theory (DFT)[134-136] with B3LYP functional[135, 137, 138] and 6-311++G(d,p) basis set. Diffused functions provide a more accurate description of

electron delocalization and are typically used as an improved treatment for cases where negatively charged species are being studied because the ionic radius of these charged atoms increases significantly as compared with the corresponding neutral examples.

To include empirical atom–atom dispersion corrections, re-optimization and quantum chemical calculations were performed for one case with long-range corrected (LC) hybrid density functional (ωB97X-D)[78] with the 6-311++G(d,p) basis set.

Harmonic vibrational frequencies were calculated at the same level of theory to characterize the stationary points by assessing the vibrational frequency of the structures in local minima. Quantum chemical descriptors including Gibbs solvation free energy (ΔG_{solv}), polarizability (α), dipole moment (μ), and $E_{HOMO-LUMO}$ gap were calculated on the optimized structure at the same level of theory. Polarizable continuum model (PCM)[139] as implemented in G09 was applied to calculate the Gibbs solvation free energy in water. This is obtained as the difference between the Gibbs free energies of the compounds in water (PCM) and vacuum (gas phase) as[140]:

$$\Delta G_{solv} = G_{water} - G_{gas} \quad (1)$$

As for the conformational study, the transitions states (TS) involved in *cis/trans* conformational changes of the considered Ac-X-OMe (X= Gly, Val, Leu, Ile, Phe, Met, Cys, Ser, Asp and His) were identified using second derivative vibrational frequency calculations throughout the scan of the amide's dihedral angle. The activation energy (E_A) is reported as the energy difference between the *trans* conformation and its transitions state and ΔE_A *cis/trans* is reported as the energy difference between *cis* and *trans* conformations. In each case, the E_A of N-methylated analogues were compared to the native amino acid series to address the impact of N-methylation on the energy barrier of *cis/trans* conformational interconversion.

More details about the calculations are presented with the supplementary material, including examples of input files, HF energies and the Cartesian coordinates of optimized structures.

Supplementary material

The frontiers orbitals (HOMO and LUMO) of more peptide derivatives, rearrangement of group charge of Val, derived from natural bond orbital analysis, Cartesian coordinates of optimized structures and transition state, 3D structures of all amino acid derivatives Ac-X-OMe (X= Gly, Asp, Ser, Val, Leu, Ile, Cys, Met, His and Phe) and their corresponding N-methylated models are provided with the supplementary materials. Two additional tables are also provided.

Acknowledgements

We thank the National Research Foundation (South Africa) and UKZN for financial support, and the CHPC (www.chpc.ac.za) for computational resources.

References

[1] Albericio F, Kruger HG. Therapeutic peptides FOREWORD. Future Medicinal Chemistry. 2012;4:1527-31.
[2] Lee DL, Hodges RS. Structure-activity relationships of de novo designed cyclic antimicrobial peptides based on gramicidin S. Biopolymers. 2003;71:28-48.
[3] Kazmierski WM, Kenakin TP, Gudmundsson KS. Peptide, Peptidomimetic and Small-molecule Drug Discovery Targeting HIV-1 Host-cell Attachment and Entry through gp120, gp41, CCR5 and CXCR4†. Chemical Biology & Drug Design. 2006;67:13-26.
[4] Eckert R, Qi FX, Yarbrough DK, He J, Anderson MH, Shi WY. Adding selectivity to antimicrobial peptides: Rational design of a multidomain peptide against Pseudomonas spp. Antimicrobial Agents and Chemotherapy. 2006;50:1480-8.
[5] Leech MD, Chung C-Y, Culshaw A, Anderton SM. Peptide-based immunotherapy of experimental autoimmune encephalomyelitis without anaphylaxis. European Journal of Immunology. 2007;37:3576-81.
[6] Wessel MD, Jurs PC, Tolan JW, Muskal SM. Prediction of Human Intestinal Absorption of Drug Compounds from Molecular Structure. Journal of Chemical Information and Computer Sciences. 1998;38:726-35.
[7] Salamat-Miller N, Johnston TP. Current strategies used to enhance the paracellular transport of therapeutic polypeptides across the intestinal epithelium. International Journal of Pharmaceutics. 2005;294:201-16.
[8] Over B, McCarren P, Artursson P, Foley M, Giordanetto F, Gronberg G, et al. Impact of Stereospecific Intramolecular Hydrogen Bonding on Cell Permeability and Physicochemical Properties. Journal of Medicinal Chemistry. 2014;57:2746-54.
[9] Kessler H. Peptoids—A New Approach to the Development of Pharmaceuticals. Angewandte Chemie International Edition in English. 1993;32:543-4.
[10] Giannis A, Kolter T. Peptidomimetics for Receptor Ligands—Discovery, Development, and Medical Perspectives. Angewandte Chemie International Edition in English. 1993;32:1244-67.
[11] Flinn N, Hussain I, Shaw A, Artursson P, Gibbons WA, Toth I. Oral absorption studies of lipid-polylysine conjugates of thyrotropin releasing hormone (TRH1) and luteinizing hormone releasing hormone (LHRH1). International Journal of Pharmaceutics. 1996;138:167-74.
[12] Fletcher MD, Campbell MM. Partially Modified Retro-Inverso Peptides: Development, Synthesis, and Conformational Behavior. Chemical Reviews. 1998;98:763-96.
[13] Haviv F, Fitzpatrick TD, Swenson RE, Nichols CJ, Mort NA, Bush EN, et al. Effect Of N-Methyl Substitution Of The Peptide-Bonds In Luteinizing-Hormone-Releasing Hormone Agonists. Journal of Medicinal Chemistry. 1993;36:363-9.

[14] Sagan S, Karoyan P, Lequin O, Chassaing G, Lavielle S. N- and C alpha-methylation in biologically active peptides: Synthesis, structural and functional aspects. Current Medicinal Chemistry. 2004;11:2799-822.
[15] Linde Y, Ovadia O, Safrai E, Xiang Z, Portillo FP, Shalev DE, et al. Structure-activity relationship and metabolic stability studies of backbone cyclization and N-methylation of melanocortin peptides. Biopolymers. 2008;90:671-82.
[16] Dong Q-G, Zhang Y, Wang M-S, Feng J, Zhang H-H, Wu Y-G, et al. Improvement of enzymatic stability and intestinal permeability of deuterohemin-peptide conjugates by specific multi-site N-methylation. Amino Acids. 2012;43:2431-41.
[17] Muppidi A, Doi K, Edwardraja S, Drake EJ, Gulick AM, Wang H-G, et al. Rational Design of Proteolytically Stable, Cell-Permeable Peptide-Based Selective Mcl-1 Inhibitors. Journal of the American Chemical Society. 2012;134:14734-7.
[18] Kessler H, Chatterjee J, Biron E, Hruby VJ, Gilon C, Hoyer D, et al. High N-methylation of peptides for improvement of activity and ADME. Journal of Peptide Science. 2006;12:94-.
[19] Biron E, Chatterjee J, Ovadia O, Langenegger D, Brueggen J, Hoyer D, et al. Improving oral bioavailability of peptides by multiple N-methylation: Somatostatin analogues. Angewandte Chemie-International Edition. 2008;47:2595-9.
[20] Ovadia O, Greenberg S, Chatterjee J, Laufer B, Opperer F, Kessler H, et al. The Effect of Multiple N-Methylation on Intestinal Permeability of Cyclic Hexapeptides. Molecular Pharmaceutics. 2011;8:479-87.
[21] Beck JG, Chatterjee J, Laufer B, Kiran MU, Frank AO, Neubauer S, et al. Intestinal Permeability of Cyclic Peptides: Common Key Backbone Motifs Identified. Journal of the American Chemical Society. 2012;134:12125-33.
[22] Wang CK, Northfield SE, Colless B, Chaousis S, Hamernig I, Lohman R-J, et al. Rational design and synthesis of an orally bioavailable peptide guided by NMR amide temperature coefficients. Proceedings of the National Academy of Sciences of the United States of America. 2014;111:17504-9.
[23] Bose PP, Chatterjee U, Hubatsch I, Artursson P, Govender T, Kruger HG, et al. In vitro ADMET and physicochemical investigations of poly-N-methylated peptides designed to inhibit Aβ aggregation. Bioorganic & Medicinal Chemistry. 2010;18:5896-902.
[24] Thansandote P, Harris RM, Dexter HL, Simpson GL, Pal S, Upton RJ, et al. Improving the passive permeability of macrocyclic peptides: Balancing permeability with other physicochemical properties. Bioorganic & Medicinal Chemistry. 2015;23:322-7.
[25] Chatterjee J, Ovadia O, Zahn G, Marinelli L, Hoffman A, Gilon C, et al. Multiple N-Methylation by a Designed Approach Enhances Receptor Selectivity. Journal of Medicinal Chemistry. 2007;50:5878-81.
[26] Fiacco SV, Roberts RW. N-Methyl Scanning Mutagenesis Generates Protease-Resistant G Protein Ligands with Improved Affinity and Selectivity. ChemBioChem. 2008;9:2200-3.
[27] Jones RE, Heimbrook DC, Huber HE, Wegrzyn RJ, Rotberg NS, Stauffer KJ, et al. Specific N-Methylations Of Hpv-16 E7 Peptides Alter Binding To The Retinoblastoma Suppressor Protein. Journal of Biological Chemistry. 1992;267:908-12.

[28] Schmidt R, KÁLmÁN A, Chung NN, Lemieux C, HorvÁTh C, Schiller PW. Structure-activity relationships of dermorphin analogues containing N-substituted amino acids in the 2-position of the peptide sequence. International Journal of Peptide and Protein Research. 1995;46:47-55.
[29] Dechantsreiter MA, Planker E, Mathä B, Lohof E, Hölzemann G, Jonczyk A, et al. N-Methylated Cyclic RGD Peptides as Highly Active and Selective αVβ3 Integrin Antagonists. Journal of Medicinal Chemistry. 1999;42:3033-40.
[30] In Y, Ishida T, Takesako K. Unique molecular conformation of aureobasidin A, a highly amide N-methylated cyclic depsipeptide with potent antifungal activity: X-ray crystal structure and molecular modeling studies. Journal of Peptide Research. 1999;53:492-500.
[31] Vogen SM, Paczkowski NJ, Kirnarsky L, Short A, Whitmore JB, Sherman SA, et al. Differential activities of decapeptide agonists of human C5a: the conformational effects of backbone N-methylation. International Immunopharmacology. 2001;1:2151-62.
[32] Siodlak D, Macedowska-Capiga A, Grondys J, Paczkowska K, Rzeszotarska B. N-Methyldehydroamino acids promote a configuration cis of N-methylamide bond. Journal of Molecular Structure-Theochem. 2008;851:100-8.
[33] Jeffs SA, Arme C. Preliminary observations on the use of alpha-N-(methylamino)isobutyric acid and 2-amino-2-norbornanecarboxylic acid to investigate amino acid transport loci in the metacestode of the rat tapeworm, Hymenolepis diminuta (Cestoda: Cyclophyllidae). Helminthologia (Bratislava). 1992;29:131-4.
[34] Gao J, Sudoh M, Aube J, Borchardt RT. Transport characteristics of peptides and peptidomimetics: I. N-methylated peptides as substrates for the oligopeptide transporter and P-glycoprotein in the intestinal mucosa. Journal of Peptide Research. 2001;57:316-29.
[35] Hughes E, Burke RM, Doig AJ. Inhibition of Toxicity in the β-Amyloid Peptide Fragment β-(25–35) Using N-Methylated Derivatives: A GENERAL STRATEGY TO PREVENT AMYLOID FORMATION. Journal of Biological Chemistry. 2000;275:25109-15.
[36] Kokkoni N, Stott K, Amijee H, Mason JM, Doig AJ. N-Methylated Peptide Inhibitors of β-Amyloid Aggregation and Toxicity. Optimization of the Inhibitor Structure†. Biochemistry. 2006;45:9906-18.
[37] Austen BM, Paleologou KE, Ali SAE, Qureshi MM, Allsop D, El-Agnaf OMA. Designing Peptide Inhibitors for Oligomerization and Toxicity of Alzheimer's β-Amyloid Peptide†. Biochemistry. 2008;47:1984-92.
[38] Bose PP, Chatterjee U, Nerelius C, Govender T, Norström T, Gogoll A, et al. Poly-N-methylated Amyloid β-Peptide (Aβ) C-Terminal Fragments Reduce Aβ Toxicity in Vitro and in Drosophila melanogaster. Journal of Medicinal Chemistry. 2009;52:8002-9.
[39] Kapurniotu A, Schmauder A, Tenidis K. Structure-based design and study of non-amyloidogenic, double N-methylated IAPP amyloid core sequences as inhibitors of IAPP amyloid formation and cytotoxicity. Journal of Molecular Biology. 2002;315:339-50.
[40] Rijkers DTS, Höppener JWM, Posthuma G, Lips CJM, Liskamp RMJ. Inhibition of Amyloid Fibril Formation of Human Amylin by N-Alkylated Amino Acid and α-Hydroxy Acid Residue Containing Peptides. Chemistry – A European Journal. 2002;8:4285-91.

[41] Tatarek-Nossol M, Yan L-M, Schmauder A, Tenidis K, Westermark G, Kapurniotu A. Inhibition of hIAPP Amyloid-Fibril Formation and Apoptotic Cell Death by a Designed hIAPP Amyloid- Core-Containing Hexapeptide. Chemistry & Biology. 2005;12:797-809.
[42] Zhao Y, Truhlar DG. A density functional that accounts for medium-range correlation energies in organic chemistry. Organic Letters. 2006;8:5753-5.
[43] Yan L-M, Velkova A, Tatarek-Nossol M, Andreetto E, Kapurniotu A. IAPP Mimic Blocks Aβ Cytotoxic Self-Assembly: Cross-Suppression of Amyloid Toxicity of Aβ and IAPP Suggests a Molecular Link between Alzheimer's Disease and Type II Diabetes. Angewandte Chemie International Edition. 2007;46:1246-52.
[44] Muthusamy K, Arvidsson PI, Govender P, Kruger HG, Maguire GEM, Govender T. Design and study of peptide-based inhibitors of amylin cytotoxicity. Bioorganic & Medicinal Chemistry Letters. 2010;20:1360-2.
[45] Tonelli AE. Effects Of Isolated N-Methylated Residues On Conformational Characteristics Of Polypeptides. Biopolymers. 1976;15:1615-22.
[46] Patel DJ, Tonelli AE. N-Methylleucine Gramicidin-S And (Di-N-Methylleucine) Gramicidin-S Conformations With Cis L-Orn-L-N-Meleu Peptide-Bonds. Biopolymers. 1976;15:1623-35.
[47] Sumner SCJ, Jiang SP, Jernigan RL, Ferretti JA. Conformational-Analysis Of Receptor Selective Tachykinin Analogs - Senktide And Septide. Journal of Biomolecular Structure & Dynamics. 1992;10:429-39.
[48] Goudreau N, Weng JH, Roques BP. Conformational-Analysis Of Cck-B Agonists Using H-1-Nmr And Restrained Molecular-Dynamics - Comparison Of Biologically-Active Boc-Trp-(N-Me)Nle-Asp-Phe-Nh2 And Inactive Boc-Trp-(N-Me)Phe-Asp-Phe-Nh2. Biopolymers. 1994;34:155-69.
[49] Bagyi I, Balogh B, Czajlik A, Elias O, Gaspari Z, Gergely V, et al. Generation and analysis of the conformational potential energy surfaces of N-acetyl-N-methyl-L-alanine-N '-methylamide. An exploratory ab initio study. Journal of Molecular Structure-Theochem. 2003;625:121-36.
[50] Sikorska E, Slusarz MJ, Slusarz R, Kowalczyk W, Lammek B. Investigation of cis/trans ratios of peptide bonds in AVP analogues containing N-methylphenylaianine enantiomers. Journal of Peptide Science. 2006;12:13-24.
[51] Chatterjee J, Mierke DF, Kessler H. Conformational preference and potential templates of N-methylated cyclic pentaalanine peptides. Chemistry-a European Journal. 2008;14:1508-17.
[52] Buczek A, Walesa R, Broda MA. beta-turn tendency in N-methylated peptides with dehydrophenylalanine residue: DFT study. Biopolymers. 2012;97:518-28.
[53] Nandel FS, Jaswal RR. Conformational study of N-methylated alanine peptides and design of A beta inhibitor. Indian Journal of Biochemistry & Biophysics. 2014;51:7-18.
[54] Piriou F, Lintner K, Fermandjian S, Fromageot P, Khosla MC, Smeby RR, et al. Amino acid side chain conformation in angiotensin II and analogs: Correlated results of circular dichroism and 1H nuclear magnetic resonance. Proceedings of the National Academy of Sciences. 1980;77:82-6.
[55] Chang CF, Zehfus MH. The effect of N-methylation on helical peptides. Biopolymers. 1996;40:609-16.
[56] Cody WL, He JX, Reily MD, Haleen SJ, Walker DM, Reyner EL, et al. Design of a Potent Combined Pseudopeptide Endothelin-A/Endothelin-B Receptor

Antagonist, Ac-dBhg16-Leu-Asp-Ile-[NMe]Ile-Trp21 (PD 156252): Examination of Its Pharmacokinetic and Spectral Properties. Journal of Medicinal Chemistry. 1997;40:2228-40.
[57] Trzepalka E, Kowalczyk W, Lammek B. Cis/trans conformational equilibrium across the N-methylphenylalanine(2)-N-methylphenylalanine(3) peptide bond of arginine vasopressin analogs. Journal of Peptide Research. 2004;63:333-46.
[58] Hughes RM, Waters ML. Influence of N-methylation on a cation-pi interaction produces a remarkably stable beta-hairpin peptide. Journal of the American Chemical Society. 2005;127:6518-9.
[59] Hughes RM, Benshoff ML, Waters ML. Effects of chain length and N-methylation on a cation-pi interaction in a beta-hairpin peptide. Chemistry-a European Journal. 2007;13:5753-64.
[60] Farahani MD, Honarparvar B, Albericio F, Maguire GEM, Govender T, Arvidsson PI, et al. Proline N-oxides: modulators of the 3D conformation of linear peptides through "NO-turns". Organic & Biomolecular Chemistry. 2014;12:4479-90.
[61] Chatterjee J, Mierke D, Kessler H. N-Methylated Cyclic Pentaalanine Peptides as Template Structures. Journal of the American Chemical Society. 2006;128:15164-72.
[62] Lameira J, Alves CN, Moliner V, Silla E. A density functional study of flavonoid compounds with anti-HIV activity. Eur J Med Chem. 2006;41:616-23.
[63] Lameira J, Medeiros IG, Reis M, Santos AS, Alves CN. Structure-activity relationship study of flavone compounds with anti-HIV-1 integrase activity: A density functional theory study. Bioorg Med Chem. 2006;14:7105-12.
[64] Wong KY, Mercader AG, Saavedra LM, Honarparvar B, Romanelli GP, Duchowicz PR. QSAR analysis on tacrine-related acetylcholinesterase inhibitors. Journal of biomedical science. 2014;21:84-.
[65] Hansch C, Quinlan JE, Lawrence GL. Linear free-energy relationship between partition coefficients and the aqueous solubility of organic liquids. The Journal of Organic Chemistry. 1968;33:347-50.
[66] Lombardo F, Shalaeva MY, Tupper KA, Gao F. ElogD(oct): A tool for lipophilicity determination in drug discovery. 2. Basic and neutral compounds. Journal of Medicinal Chemistry. 2001;44:2490-7.
[67] Pajouhesh H, Lenz GR. Medicinal chemical properties of successful central nervous system drugs. NeuroRx : the journal of the American Society for Experimental NeuroTherapeutics. 2005;2:541-53.
[68] Remko M. Acidity, lipophilicity, solubility, absorption, and polar surface area of some ACE inhibitors. Chemical Papers. 2007;61:133-41.
[69] Masao Fujisawaa, Hirotaka Tsutsumia, Takayoshi Kimura. Prediction of solubility of practically insoluble drugs in water/ethanol solvents using non-empirical methods. J Chem Pharm Res. 2011;3(3):750-8.
[70] Monajjemi M, Khosravi M, Honarparvar B, Mollaamin F. Substituent and Solvent Effects on the Structural Bioactivity and Anticancer Characteristic of Catechin as a Bioactive Constituent of Green Tea. International Journal of Quantum Chemistry. 2011;111:2771-7.
[71] Sarasia EM, Afsharnezhad S, Honarparvar B, Mollaamin F, Monajjemi M. Theoretical study of solvent effect on NMR shielding tensors of luciferin derivatives. Physics and Chemistry of Liquids. 2011;49:561-71.

[72] Sarasia EM, Soliman MES, Honarparvar B. Theoretical study on the molecular electronic properties of salicylic acid derivatives as anti-inflammatory drugs. Journal of Structural Chemistry. 2012;53:574-81.
[73] Honarparvar B, Govender T, Maguire GEM, Soliman MES, Kruger HG. Integrated Approach to Structure-Based Enzymatic Drug Design: Molecular Modeling, Spectroscopy, and Experimental Bioactivity. Chemical Reviews. 2014;114:493-537.
[74] Domańska U, Pobudkowska A, Pelczarska A, Winiarska-Tusznio M, Gierycz P. Solubility and pKa of select pharmaceuticals in water, ethanol, and 1-octanol. The Journal of Chemical Thermodynamics. 2010;42:1465-72.
[75] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews. 1997;23:3-25.
[76] Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. Journal of Medicinal Chemistry. 2002;45:2615-23.
[77] Savjani KT, Gajjar AK, Savjani JK. Drug solubility: importance and enhancement techniques. ISRN pharmaceutics. 2012;2012:195727-.
[78] Babu S, Sohn H, Madhavan T. Computational Analysis of CRTh2 receptor antagonist: A Ligand-based CoMFA and CoMSIA approach. Computational Biology and Chemistry. 2015;56:109-21.
[79] Drew KLM, Reynisson J. The impact of carbon-hydrogen bond dissociation energies on the prediction of the cytochrome P450 mediated major metabolic site of drug-like compounds. European Journal of Medicinal Chemistry. 2012;56:48-55.
[80] Ioakimidis L, Thoukydidis L, Mirza A, Naeem S, Reynisson J. Benchmarking the reliability of QikProp. Correlation between experimental and predicted values. Qsar & Combinatorial Science. 2008;27:445-56.
[81] Lameira J, Alves CN, Moliner V, Silla E. A density functional study of flavonoid compounds with anti-HIV activity. European Journal of Medicinal Chemistry. 2006;41:616-23.
[82] Lameira J, Medeiros IG, Reis M, Santos AS, Alves CN. Structure-activity relationship study of flavone compounds with anti-HIV-1 integrase activity: A density functional theory study. Bioorganic & Medicinal Chemistry. 2006;14:7105-12.
[83] Molina E, Estrada E, Nodarse D, Torres LA, Gonzalez H, Uriarte E. Quantitative structure-antibacterial activity relationship modeling using a combination of piecewise linear regression-discriminant analysis (I): Quantum chemical, topographic, and topological descriptors. International Journal of Quantum Chemistry. 2008;108:1856-71.
[84] Thillainayagam M, Pandian L, Murugan KK, Vijayaparthasarathi V, Sundaramoorthy S, Anbarasu A, et al. In silico analysis reveals the anti-malarial potential of quinolinyl chalcone derivatives. Journal of Biomolecular Structure & Dynamics. 2015;33:961-77.
[85] Aihara J-i. Correlation found between the HOMO-LUMO energy separation and the chemical reactivity at the most reactive site for isolated-pentagon isomers of fullerenes. Physical Chemistry Chemical Physics. 2000;2:3121-5.

[86] Cao CZ, Li ZL. Molecular polarizability. 1. Relationship to water solubility of alkanes and alcohols. Journal of Chemical Information and Computer Sciences. 1998;38:1-7.
[87] Li Feng-Yu, Zhao Ji-Jun Quantum chemistry PM3 calculations of sixteen mEGF molecules. 2010;1:68-77.
[88] Abraham MH, Le J. The correlation and prediction of the solubility of compounds in water using an amended solvation energy relationship. Journal of Pharmaceutical Sciences. 1999;88:868-80.
[89] Hatua K, Nandi PK. Relationships Between Different-Order Polarizabilities And Ground State Dipole Moment (Vol 12, 1250099, 2013). Journal of Theoretical & Computational Chemistry. 2013;12.
[90] Lin T-Y, Chaudhari A, Lee S-L. Correlation between substituent constants and hyperpolarizabilities for di-substituted trans-azobenzenes. Journal of Molecular Modeling. 2013;19:529-38.
[91] Blair SA, Thakkar AJ. Relating polarizability to volume, ionization energy, electronegativity, hardness, moments of momentum, and other molecular properties. Journal of Chemical Physics. 2014;141.
[92] Kruger HG. Ab initio mechanistic study of the protection of alcohols and amines with anhydrides. Journal of Molecular Structure-Theochem. 2002;577:281-5.
[93] Tajkhorshid E, Suhai S. Influence of the Methyl Groups on the Structure, Charge Distribution, and Proton Affinity of the Retinal Schiff Base. The Journal of Physical Chemistry B. 1999;103:5581-90.
[94] Dumont RS. Effects of charging and polarization on molecular conduction via the source-sink potential method. Canadian Journal of Chemistry-Revue Canadienne De Chimie. 2014;92:100-11.
[95] Jung IH, Lo W-Y, Jang J, Chen W, Zhao D, Landry ES, et al. Synthesis and Search for Design Principles of New Electron Accepting Polymers for All-Polymer Solar Cells. Chemistry of Materials. 2014;26:3450-9.
[96] Gallagher SH, Armstrong RS, Lay PA, Reed CA. SOLVENT EFFECTS ON THE ELECTRONIC-SPECTRUM OF C-60. Journal of Physical Chemistry. 1995;99:5817-25.
[97] Makovskaya V, Dean JR, Tomlinson WR, Comber M. OCTANOL-WATER PARTITION-COEFFICIENTS OF SUBSTITUTED PHENOLS AND THEIR CORRELATION WITH MOLECULAR DESCRIPTORS. Analytica Chimica Acta. 1995;315:193-200.
[98] Farah JPS, Lima GAR, Quina FH. Using quantum chemistry to predict solubilization in detergent micelles. Theochem-Journal of Molecular Structure. 1997;394:267-70.
[99] Yin CS, Liu XH, Guo WM, Lin T, Wang XD, Wang LS. Prediction and application in QSPR of aqueous solubility of sulfur-containing aromatic esters using GA-based MLR with quantum descriptors. Water Research. 2002;36:2975-82.
[100] Pearson RG. Absolute electronegativity and hardness: applications to organic chemistry. The Journal of Organic Chemistry. 1989;54:1423-30.
[101] Diener MD, Alford JM. Isolation and properties of small-bandgap fullerenes. Nature. 1998;393:668-71.

[102] Pearson RG. ABSOLUTE ELECTRONEGATIVITY AND HARDNESS - APPLICATIONS TO ORGANIC-CHEMISTRY. Journal of Organic Chemistry. 1989;54:1423-30.
[103] Jeyavijayan S. Molecular structure, spectroscopic (FTIR, FT-Raman, C-13 and H-1 NMR, UV), polarizability and first-order hyperpolarizability, HOMO-LUMO analysis of 2,4-difluoroacetophenone. Spectrochimica Acta Part a-Molecular and Biomolecular Spectroscopy. 2015;136:553-66.
[104] Suresh S, Gunasekaran S, Srinivasan S. Vibrational spectra (FT-IR, FT-Raman), frontier molecular orbital, first hyperpolarizability, NBO analysis and thermodynamics properties of Piroxicam by HF and DFT methods. Spectrochimica Acta Part a-Molecular and Biomolecular Spectroscopy. 2015;138:447-59.
[105] Lewis FD, Long TM, Stern CL, Liu WZ. Structures and excited states of extended and folded mono-, di-, and tri-N-arylbenzamides. Journal of Physical Chemistry A. 2003;107:3254-62.
[106] Helbing J, Bregy H, Bredenbeck J, Pfister R, Hamm P, Huber R, et al. A fast photoswitch for minimally perturbed peptides: Investigation of the trans -> cis photoisomerization of N-methylthioacetamide. Journal of the American Chemical Society. 2004;126:8823-34.
[107] Szabo Attila. , Ostlund Neil.S. Modern Quantum Chemistry: Introduction to Advanced Electronic Structure Theory. Mineola, New York: McGraw Hill; 1989.
[108] Wheland GW. Resonance in Organic Chemistry. New York: Wiley; 1955.
[109] Pauling L. The Nature of the Chemical Bond; . Ithaca, NY.: Cornell University Press: Cornell; 1960.
[110] Wiberg KB, Laidig KE. Barriers To Rotation Adjacent To Double-Bonds .3. The C-O Barrier In Formic-Acid, Methyl Formate, Acetic-Acid, And Methyl Acetate - The Origin Of Ester And Amide Resonance. Journal of the American Chemical Society. 1987;109:5935-43.
[111] Wiberg KB, Breneman CM. Resonance Interactions In Acyclic Systems .3. Formamide Internal-Rotation Revisited - Charge And Energy Redistribution Along The C-N Bond Rotational Pathway. Journal of the American Chemical Society. 1992;114:831-40.
[112] Laidig KE, Cameron LM. Barrier to rotation in thioformamide: Implications for amide resonance. Journal of the American Chemical Society. 1996;118:1737-42.
[113] MilnerWhite EJ. The partial charge of the nitrogen atom in peptide bonds. Protein Science. 1997;6:2477-82.
[114] Mujika JI, Matxain JM, Eriksson LA, Lopez X. Resonance structures of the amide bond: The advantages of planarity. Chemistry-a European Journal. 2006;12:7215-24.
[115] Kemnitz CR, Loewen MJ. "Amide resonance" correlates with a breadth of C-N rotation barriers. Journal of the American Chemical Society. 2007;129:2521-8.
[116] Yamada K, Koga N. Variationally determined electronic states for the theoretical analysis of intramolecular interaction: I. Resonance energy and rotational barrier of the C-N bond in formamide and its analogs. Theoretical Chemistry Accounts. 2012;131.
[117] Alabugin IV, Gilmore KM, Peterson PW. Hyperconjugation. Wiley Interdisciplinary Reviews-Computational Molecular Science. 2011;1:109-41.

[118] Frisch MJ, Pople JA, Binkley JS. Self‐consistent molecular orbital methods 25. Supplementary functions for Gaussian basis sets. The Journal of Chemical Physics. 1984;80:3265-9.
[119] Arnaud R. Ab-Initio Study Of Some Ch(3)Ocxych(2) Radicals - The Influence Of Anomeric Effects On Their Structure And Their Stability. Journal of Computational Chemistry. 1994;15:1341-56.
[120] Kwon O, Sevin F, McKee ML. Density functional calculations of methyllithium, t-butyllithium, and phenyllithium oligomers: Effect of hyperconjugation on conformation. Journal of Physical Chemistry A. 2001;105:913-22.
[121] Martins-Costa M, Anglada JM, Ruiz-Lopez MF. Hyperconjugation in adjacent OO bonds: Remarkable odd/even effects. Chemical Physics Letters. 2009;481:180-2.
[122] Greenway KT, Bischoff AG, Pinto BM. Probing Hyperconjugation Experimentally with the Conformational Deuterium Isotope Effect. Journal of Organic Chemistry. 2012;77:9221-6.
[123] Kessler H. Detection of Hindered Rotation and Inversion by NMR Spectroscopy. Angewandte Chemie International Edition in English. 1970;9:219-35.
[124] Goodman M, Chen F, Lee C-Y. Conformational aspects of polypeptide structure. XLV. Nuclear magnetic resonance study of trans-cis isomerization in an N-methyl-L-alanine derivative. Journal of the American Chemical Society. 1974;96:1479-84.
[125] Laufer B, Chatterjee J, Frank AO, Kessler H. Can N-methylated amino acids serve as substitutes for prolines in conformational design of cyclic pentapeptides? Journal of Peptide Science. 2009;15:141-6.
[126] Laufer B, Frank AO, Chatterjee J, Neubauer T, Mas-Moruno C, Kummerlöwe G, et al. The Impact of Amino Acid Side Chain Mutations in Conformational Design of Peptides and Proteins. Chemistry – A European Journal. 2010;16:5385-90.
[127] Leis J, Klika KD, Karelson M. Solvent polarity effects on the E/Z conformational equilibrium of N-1-naphthylamides. Tetrahedron. 1998;54:7497-504.
[128] Zhang J, Germann MW. Characterization of Secondary Amide Peptide Bond Isomerization: Thermodynamics and Kinetics from 2D NMR Spectroscopy. Biopolymers. 2011;95:755-62.
[129] Henrickson JB, Cram DJ, Hammond GS. Organic Chemistry. New York: McGraw-Hill; 1970.
[130] Budesinsky M, Symersky J, Jecny J, Vanhecke J, Hosten N, Anteunis M, et al. Cyclo(L-Prolyl-L-N-Methylphenylalanyl) - Conformation In Solution And In The Crystal. International Journal of Peptide and Protein Research. 1992;39:123-30.
[131] Magzoub M, Eriksson LEG, Graslund A. Conformational states of the cell-penetrating peptide penetratin when interacting with phospholipid vesicles: effects of surface charge and peptide concentration. Biochimica Et Biophysica Acta-Biomembranes. 2002;1563:53-63.
[132] ACD/ChemSketch. version 12.01,Advanced Chemistry Development, Inc. Toronto, ON, Canada2013.
[133] Gaussian 09 RA, Frisch MJT, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, Jr., J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.;

Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; and Pople, J. A.;. Gaussian, Inc., Wallingford CT. 2009.

[134] Becke AD. Density-functional exchange-energy approximation with correct asymptotic behavior. Physical Review A. 1988;38:3098-100.

[135] Parr RG, Yang W. Density Functional Theory of Atoms and Molecules. Oxford, UK: Oxford University Press; 1989.

[136] Burke K. Perspective on density functional theory. Journal of Chemical Physics. 2012;136:150901-9.

[137] Perdew JP, Chevary JA, Vosko SH, Jackson KA, Pederson MR, Singh DJ, et al. Erratum: Atoms, molecules, solids, and surfaces: Applications of the generalized gradient approximation for exchange and correlation. Physical Review B. 1993;48:4978-.

[138] Perdew JP, Burke K, Wang Y. Generalized gradient approximation for the exchange-correlation hole of a many-electron system. Physical Review B. 1996;54:16533-9.

[139] Tomasi J, Mennucci B, Cammi R. Quantum mechanical continuum solvation models. Chemical Reviews. 2005;105:2999-3093.

[140] Mananghaya M, Rodulfo E, Santos GN, Villagracia AR, Ladines AN. Theoretical Investigation on Single-Wall Carbon Nanotubes Doped with Nitrogen, Pyridine-Like Nitrogen Defects, and Transition Metal Atoms. Journal of Nanomaterials. 2012.