COMMUNICATION

Porous Molecular Materials from Hybrid Dipeptides Containing Non-proteinogenic Residues

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Abstract: In our search to fabricate novel porous molecular crystals of biological or biomimetic origin, we employed proteinogenic and non-proteinogenic hydrophobic amino acids to construct ten hybrid dipeptidic materials with open porosity. The combination of single-crystal XRD, solid-state NMR and conformational analysis revealed their crystal structure, the conformational arrangements and dynamics of the side chains. The structures display empty helical nanochannels with a cross-section from 2.3 to 5.1 Å modulated by the size and shape of the hydrocarbon side chains. The self-assembly of L-2-aminobuturyl-L-2-aminobutyric acid (L-Abu-L-Abu) shows the largest channels observed for this class of dipeptides. Crystal pore accessibility from the gas phase was monitored by CO_2 and CH_4 adsorption isotherms: included CO_2 was spectroscopically detected by 2D MAS NMR.

The molecular self-assembly of peptides has long been an area of interest in structural, biological and material sciences.[1] In recent years assemblies of peptides derived from proteinogenic (natural) amino acid residues have become a new source of porous bio-organic materials.[2] Indeed, in the strategy to develop environmentally friendly green materials, such porous crystalline substances have attracted considerable attention for their potential as efficient absorbents for both green-house gases and other harmful gases.[3] Moreover, the bio-compatibility of peptides suggests possible applications in biomedicine and their use as drug delivery systems.[4]

The development of porous dipeptides was triggered by the discovery of channels in the crystal structure of L-valyl-L-alanine (Val-Ala),[5] which stimulated a series of investigations using natural dipeptides with two hydrophobic residues. Two families of structures were revealed: the Phe-Phe class with hydrophilic channels, based on combinations of amino acid residues with bulky side groups such as Leu, Phe and Trp, and the Val-Ala class obtained from combinations of Ala, Val and Ile with pendant alifatic groups.[2a] Prior to the present investigation, there were only seven known members of the latter family, which is unique by virtue of the fact that the guests within the channels can be removed, resulting in a permanently porous crystal structure. This, in turn, renders the empty channels available to adsorb a variety of guests, with full retention of the host structure.[6] However, all known members of the nanoporous Val-Ala class are composed of standard proteinogenic amino acid residues,[2a] which impose clear limitations on their strategic use for structural diversification. Therefore, in the strive to fabricate novel porous molecular materials, an objective of intense interest at the present time,[2,7] we decided to explore an expansion of this family of hydrophobic dipeptides by incorporating the non-proteinogenic amino acids L-aminobutyric acid (Abu), with an ethyl side chain, and L-norvaline (Nva) with n-propyl side chain. Although not derived from natural sources, such amino acids are biocompatible and enviromentally friendly. Indeed, physiological testing has shown that Nva is an arginase inhibitor, thus regulating the nitric oxide (NO) production in mammals and acting as an anti-inflammatory agent,[8] while Abu is an active precursor used in both anticonvulsant drug molecules and antituberculotic drugs.[9] This demonstrates the non-hazardous use of Abu and Nva residues as building blocks for the construction of bio-organic and biocompatible porous materials potentially useful as drug carriers.

Here we present the synthesis and characterization of ten novel hybrid dipeptide structures **1**-**10** (Figure 1) constructed from a combination of proteinogenic and non-proteinogenic amino acids residues. They were crystallized in permanently porous architectures forming parallel and independent nanochannels. The key feature of these structures is the presence of chiral channels with distinct diameters and helicities, modulated by the encumbrance of the lateral substituents that point towards the channel axes. Structure determinations were provided by single crystal X-ray diffraction analysis, while the accessibility and intimacy of CO_2 with the matrix was proven by adsorption isotherms and by direct 1D and 2D MAS NMR detection.

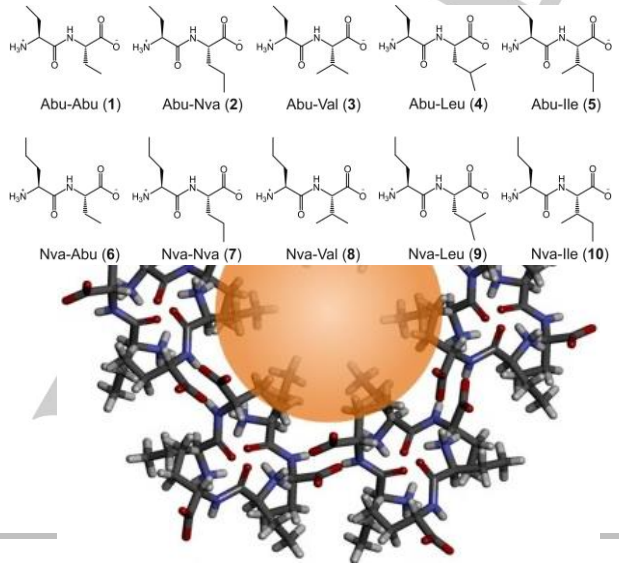
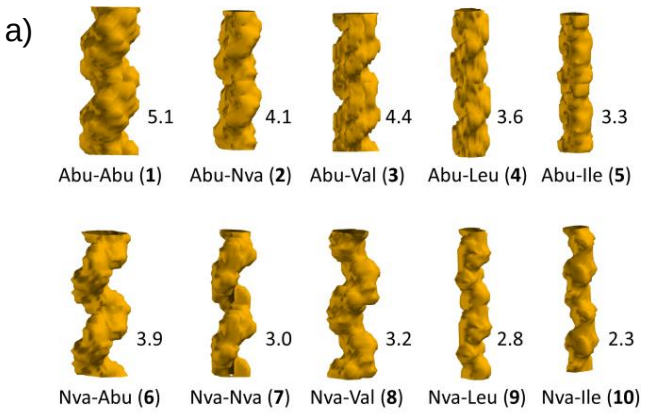


Figure 1. Chemical structures of the ten dipeptides (above) and the crystal structure of Abu-Abu **1** (below).

The synthesis of the ten dipeptides (**1**-**10**) is reported in Supporting Information. Single crystals were obtained by gel crystallization vapor diffusion techniques (acetonitrile vapors), and subsequent treatment under vacuum at 80°C producing the porous crystals. The structural parameters confirmed that all the compounds belong to the space groups $P6_1$, and form one-dimensional homochiral channels (Supporting Information). No electron density along the channels was detected. The porous crystals are sustained by a unique hydrogen bonding pattern with left-handed double helices of head-to-tail chains with three-fold screw symmetry. Amino···carbonyl interactions link the helix strands, while hydrogen bonds to the carboxylate groups, with amino, amide and C^{α}−H donors form connections to three neighboring double helices and, generate a robust supramolecular network. Remarkably, Abu-Leu (**4**) and Nva-Leu (**9**) are members of the present family of porous dipeptides, although we had previously postulated that dipeptides containing Leu residues were incompatible with the formation of porous structures due to steric conflict between the Leu side chain and the amino group of an adjacent peptide.[2a,10] Indeed, we now note that the associated short intermolecular H···H distances can be alleviated in the class of hybrid dipeptides by small changes of main-chain and side-chain torsion angles.

The channels of crystal structures **1**-**10** are illustrated in Figure 2. They exhibit the shape of a right-handed helix, with their grooves carved by the terminal methyl groups of the peptide side chains. Abu-Abu (**1**) forms pores with a diameter of 5.1 Å and has a void volume of 307 $Å^3$ (17.4 % of the unit cell volume), which is comparable to the channels of Val-Ala and Ala-Val. While Val-Ala is conformationally locked with respect to side chain orientation, Abu-Abu, with the same number of side chain carbon atoms (two ethyl groups vs. isopropyl + methyl groups) can choose from five different combinations of side chain rotamers (Supporting Information). Thus, the channel shape and diameter of Abu-Abu is tunable, and the absorption of large guests can trigger conversion to other conformations that allow the channel diameter to reach values close to 6 Å. The 3.3 Å pore size of Abu-Ile (**5**) is, surprisingly, smaller than 3.7 Å of Val-Ile. This observation may seem counterintuitive, as the Abu-to-Val substitution means replacing a H atom with a methyl group, but it reflects the substantial increase in unit cell volume, from 1840.5(6) $Å^3$ for **5** to 1940.8(4) $Å^3$ for Val-Ile, which forms channels with a higher helicity than **5**. For the corresponding pair Abu-Val (**3**) and Val-Val the channel diameter remains unchanged at 4.4 Å.

The side chain of the *N*-terminal Nva of peptides **6**–**10** has an extra methyl group compared to the *N*-terminal Abu for **1**-**5**, resulting in a substantial reduction in channel cross-sections and diameters. Nva-Ile (**10**) shows the smallest cross section of all the dipeptides, 2.3 Å, considerably smaller than 3.7 Å and 3.9 Å of Val-Ile and its retroanalogue Ile-Val, respectively. Overall, there is an inverse correlation between side chain encumbrance and the channel diameter, but with substantial variations depending on the specific residues involved, as apparent from the plot in Figure 2. The crystal packing arrangements of peptides **1**-**10** have remarkably low density: among the compounds with formula $C_8H_{16}N_2O_3$ (a total of four C atoms in the side chains), Abu-Abu (**1**) displays a crystal density of 1.06 g/cm^3, which is the benchmark of the family, while in the $C_9H_{18}N_2O_3$ series the densities of 1.11 g/cm^3 in Abu-Nva, Abu-Val and Nva-Abu are lower than that of Ile-Ala (1.15 g/cm^3).



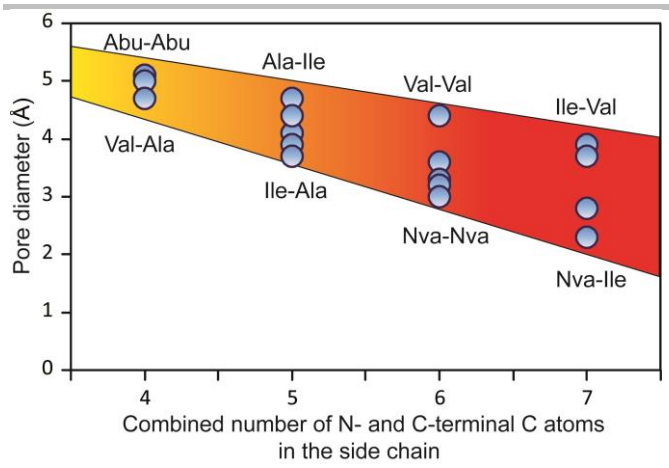


Figure 2. a) Channel-like pore volumes of the **1-10** crystals as explored by a sphere of 1.2 Å radius. (1.0 Å for **10**) and 0.5 Å grid spacing. On the right side of the channels, the corresponding cross-sections are reported (Å). b) Correlation between side chain encumbrance and pore diameter for the seventeen members of the Val-Ala class. Compounds representing extreme values within each group have been indicated.

The regular dipeptides of the Val-Ala class incorporate, in addition to Ala, Val and Ile residues with branched side-chains of limited conformational freedom. In contrast, the more unrestricted ethyl and propyl side chains of the new residues, Abu and Nva, suggest that a diversity of side-chain conformations may be explored, resulting in a soft alifatic layer lining the channel walls. This intriguing feature was addressed by a comprehensive conformational analysis of all the 17 dipeptides prepared so far (including the 7 natural ones) as well as 8 potential, as yet uninvestigated, members of the Val-Ala class. The results of this investigation (Supporting material), confirm that a peptide like Val-Val has but one conformation compatible with the hexagonal space group, while Abu-Abu (**1**) and Nva-Abu (**6**) have no less than five viable conformations. In peptides **6**–**10**, the side chain of the *N*-terminal Nva residues is disordered over two conformations with χ_1^2 = *trans* or *gauche*$^-$, Figure 3. The reason for this disorder is that the energetically preferred conformation with χ_1^2 = *trans*[1] leads to prohibitively short contacts at the center of the channel. Instead, this steric conflict between adjacent molecules is relieved when side chains are arranged in alternating *trans* and *gauche*$^-$ conformations. The resulting channels have local three-fold screw symmetry in the hexagonal unit cell. Structures with *N*-terminal Nva were also refined (to higher *R*-factors) in the trigonal space group $P3_1$ for calculations of void volumes.

^{13}C Solid state MAS NMR spectroscopy is very sensitive to conformations and motional behavior of alkyl chains, and was effective to give an insight into the arrangement and mobility of the dipeptide side chains in the crystals.

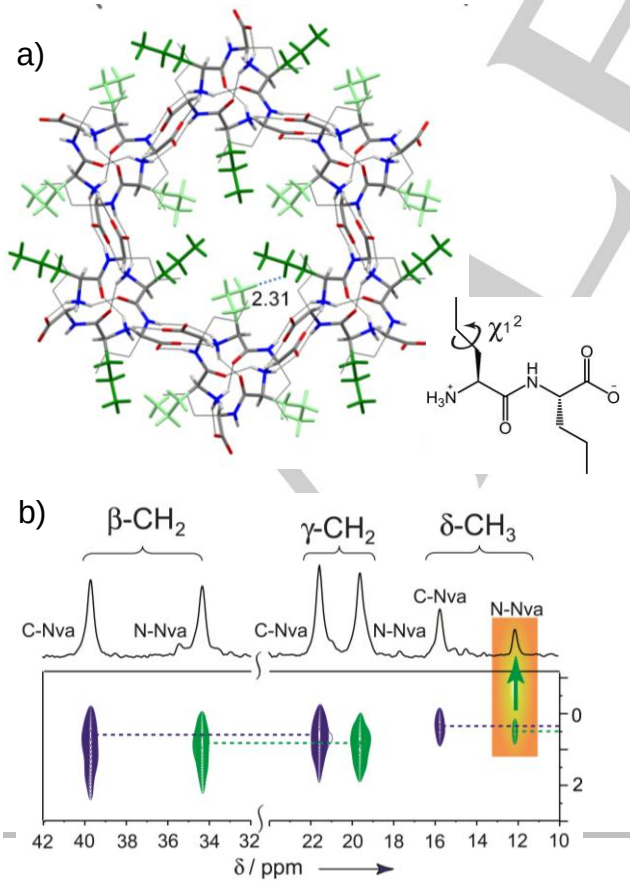


Figure 3. a) Crystal structure of Nva-Nva (**7**) showing the alternated trans gauche conformations of the propyl side chains. In the inset, the chemical structure of Nva-Nva is shown. b) 2D ^{1}H-^{13}C NMR spectrum of Nva-Nva (**7**): expansion of the aliphatic region. The methyl group of the N-terminal Nva residue is highlighted in yellow.

In the 2D ^{1}H-^{13}C HETCOR MAS NMR spectra of Nva-Nva, Nva-Val and Nva-Ile we observed signals, due to the pendant groups, in the aliphatic region (from 10 to 40 ppm on the carbon domain), in which the methyl groups of the *N*-terminal Nva residue resonate at δ_C= 12.5 12.3 and 13.0 ppm, respectively (Figure 3). Such high-field resonances are typical of *n*-alkane chain-end methyls, experiencing the effect of *gauche* conformations of the vicinal -CH_2-CH_2– bond (γ-gauche effect).[12] Since the *trans*-to-*gauche* conversion accounts for Δ= -5 ppm in hydrocarbons, the observed CH_3 chemical shifts of about 3 ppm upfield with respect to the expected *trans* conformation resonance, indicates that at least a 0.5 fraction of *gauche* conformation is explored, consistent with XRD data. Spin-lattice relaxation times from 0.3 to 1.7 s for Nva alkyl pendant group are close to the theoretical minimum value, indicating an efficient relaxation mechanism of about 10^8 Hz. Consequently, in the family of dipeptides with *N*-terminal Nva residues a fast dynamic exchange between conformations occurs, realizing an intriguing case of fast dynamics in molecular crystals, which has been generally achieved by the insertion of engineered mobile elements.[13] Indeed, in the present instance, dynamics is attained unconventionally by the generation of a channel-like free space in the crystalline structure, which promotes the mobility of the moieties that protrude towards the pores. Furthermore, no guests occluding the channels were found by both ^{1}H and ^{13}C MAS NMR, in agreement with the lack of residual electron density along the channels in the refined crystal structures.

This available space can be exploited for accomodating guests diffusing in, provided the crystal channels are open towards the gas phase and no restrictions occur on the (001) crystal surface. The open and permanent porosity of the new hybrid dipeptides was demonstrated by adsorption isotherms of gases. The isotherms of CO_2 and CH_4 for a few representative dipeptides at 195K and 1 bar are reported in Figure 4.

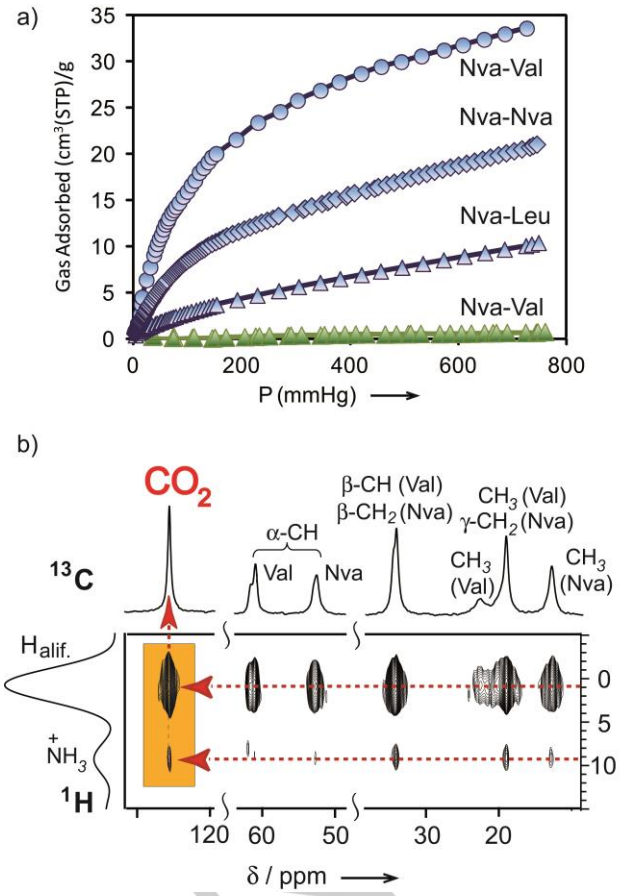


Figure 4. a) CO_2 and CH_4 adsorption isotherms of dipeptides Nva-Val, Nva-Nva and Nva-Leu at 195K and up to 1 bar. CO_2 and CH_4 values are indicated by blue and green labels, respectively. b) 2D ^{1}H-^{13}C MAS NMR of Nva-Val loaded with ^{13}C-enriched CO_2, as recorded at 240 K. In the hydrogen domain the trace of CO_2 resonance and in the carbon domain the 1D ^{13}C CP MAS spectrum are reported.

The CO_2 curve of dipeptide **8** (Nva-Val) shows a type I Langmuir behavior that is typical of microporous systems, Nva-Val can adsorb 35 cm^3(STP)/g of CO_2 at 195K and 1 bar, which corresponds to 2 molecules per unit cell. Considering the van der Waals' volume of the CO_2 molecule, a virtually complete filling of the available structural voids is achieved. The carbon dioxide isotherms for dipeptides **7** and **9** (Nva-Nva and Nva-Leu, respectively) follow a parallel behavior with lower sorption capacities. The higher capacity of Nva-Val

is consistent with the larger volume available, realized by the channel helicity that develops in the crystalline structure and its larger channel cross section (3.2 Å), compared to the smaller diameters of dipeptides Nva-Nva and Nva-Leu (3.0 and 2.8 Å, respectively). As a matter of fact, the Nva-Val > Nva-Nva > Nva-Leu trend for the adsorbed CO_2 amount at 1 bar mirrors the available unit cell void volumes at 152, 122 and 82 $Å^3$, respectively. Notably, the dipeptide crystals show an extremely low amount of adsorbed CH_4 under the same pressure and temperature conditions, due to the excessive size of CH_4 molecule (4.4 Å vdW diameter) compared to the channel sizes, and the selective absorption of CO_2 over CH_4 reaches a ratio of more than 50 times at 1 bar in Nva-Val. The isosteric heat of CO_2 adsorption was measured to be 20 kJ/mol at low coverage (as derived by Clausius-Clapeyron equation), revealing the formation of weak interactions of CO_2 with aliphatic groups, i.e. methyls and *n*-propyl groups, which protrude from the dipeptide molecules and line the inner surface of the channel walls.

The direct observation of CO_2 molecules inside the channels was provided by 1D and 2D ^1H–^{13}C MAS NMR experiments with cross-polarization from hydrogen to carbon nuclei. Cross-polarization is effective between hydrogens and carbons that experience mutual dipole-dipole interactions and reside at close distances (within a few Angstroms) for a residence time long enough to allow the magnetization transfer (in the order of milliseconds). This phenomenon is particularly interesting when the through-space interaction involves gas-solid interfaces. Indeed, when recorded at low temperature (240 K), the ^{13}C CP MAS NMR spectrum of Nva-Val loaded with ^{13}C-enriched CO_2 exhibits an intense signal at δ_C=125.6 ppm due to CO_2 adsorbed in the nanochannels (Figure 4b). As CO_2 does not possess hydrogens, the sole source of magnetization is that conveyed from host hydrogens to CO_2 carbons in the surrounding space. Moreover, 2D ^1H–^{13}C heterocorrelated HETCOR experiments of Nva–Val/CO_2 show correlated signals: an indication that close-contact interactions of the host alifatic hydrogens with CO_2 carbons occur as strongly as with their covalently bonded carbons. Notably, CO_2 also correlates with ammonium hydrogens. Such heterocorrelated experiments unequivocally prove the intimacy of the adsorbed gas and the porous solid, since correlation intensity diminishes rapidly with distance (*d*), according to the function $1/d^6$.

In conclusion, the use of non-proteinogenic amino acid residues[2] allowed us to generate ten new crystal structures of hydrophobic dipeptides showing permanent porosity.[14] The diversification of the amino acid building blocks substantially enriches the Val-Ala class, until now limited to compounds with proteinogenic residues, and allows fine-tuning of channel cross-sections by a systematic change of the hydrophobic side chains. In fact, the space available within the channels is modulated by the encumbrance and rotational freedom of the protruding side chains. Adsorption isotherms at variable temperature demonstrate the availability of the crystalline channels to diffusing gases, showing a preferable CO_2 uptake over CH_4. The present achievements open the way to expand the search for nanoporous dipeptides containing other non-proteogenic amino acids with even more untraditional side chains, e.g. peptides like FAla-Abu and Abu-Fala, where FAla is fluoroalanine with a CFH_2 side chain, would be extremely stimulating as such structures would potentially enlarge the pore volume and change the nature of the channel walls. As the non-proteinogenic amino acids adopted here (Nva and Abu) in combination with those of natural origin, are non-hazardous and in use as active compounds for medical purposes, it can be envisaged that the new hybrid dipeptides could be applied for drug delivery and to promote interactions with biological functions.

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Keywords: Crystal Engineering • Microporous materials • Peptides • Absorption • X-ray diffraction

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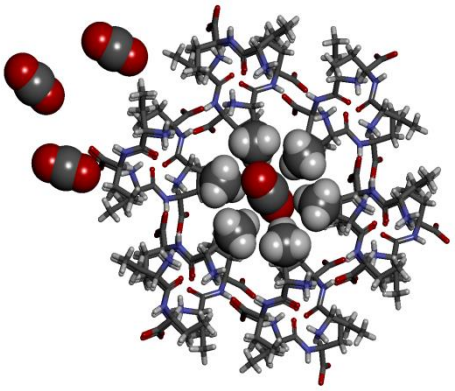
[14] CCDC 995070 - 995079 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Entry for the Table of Contents

COMMUNICATION

Crystals of dipeptides constituted by a combination of proteinogenic and non-proteinogenic residues show open nanochannels with variable diameters modulated by the encumbrance of the latter units. The accessibility of these novel porous materials from the gas-phase was demonstrated by adsorption isotherms of CO_2 and CH_4, which indicate selective uptake of CO_2. Direct observations of captured CO_2 in 1D and 2D NMR spectra revealed gas-matrix atomic-scale interaction.



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