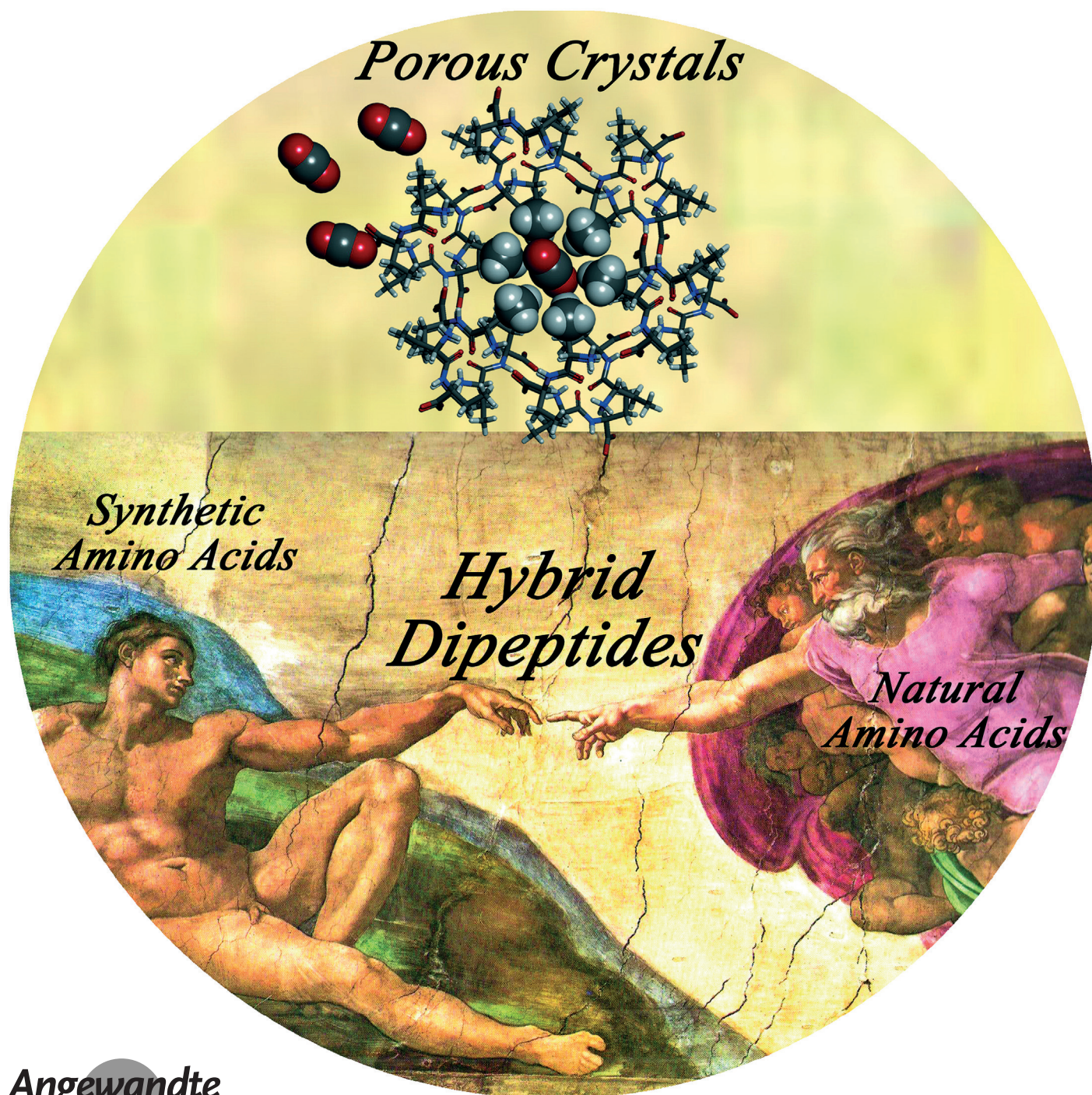


Microporous Molecular Materials from Dipeptides Containing Non-proteinogenic Residues

Vitthal N. Yadav, Angiolina Comotti,* Piero Sozzani, Silvia Bracco, Tore Bonge-Hansen, Martin Hennum, and Carl Henrik Görbitz*



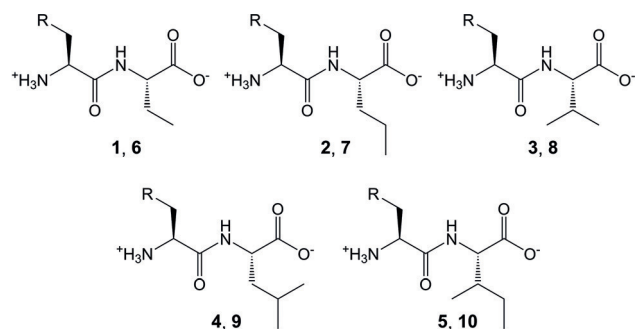
Abstract: Dipeptides with two hydrophobic side chains have proved to be an exceptional source of microporous organic materials, but since previous structures were limited to the incorporation of only proteinogenic residues, their full potential as adsorbents has remained unexplored. Single-crystal XRD data for ten new compounds with non-proteinogenic L-2-aminobutanoic acid and/or L-2-amino-pentanoic acid are presented. The gas-phase accessibility of their crystal pores, with cross-sections of 2.3 to 5.1 Å, was monitored by CO₂ and CH₄ adsorption isotherms. Included CO₂ was also detected spectroscopically by 2D MAS NMR. An extensive conformational analysis reveals that the use of linear rather than branched side chains (such as L-valine and L-isoleucine) affords peptides with a greater degree of conformational freedom and yields more-flexible channel surfaces that may easily adapt to a series of potential guest molecules.

The molecular self-assembly of peptides has long been an area of interest in structural, biological, and materials sciences.^[1] In recent years, assemblies of peptides derived from proteinogenic (natural) amino acid residues have become a new source of porous bioorganic materials.^[2] In the endeavor 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 biocompatibility of peptides suggests possible applications in biomedicine and their use as drug delivery systems.^[4]

Dipeptides with two hydrophobic residues are the smallest molecules that form microporous crystals. The initial discovery of channels in L-valyl-L-alanine (Val-Ala)^[5] stimulated a series of investigations that revealed that it was the first member of a group of related structures (the Val-Ala class) obtained from combinations of Ala, Val, and Ile. A second group, called the Phe-Phe class, has hydrophilic channels and is based on residues with bulky side groups such as Leu, Phe, and Trp.^[2a] The Val-Ala class is unique by virtue of the fact that co-crystallized solvent molecules within the channels can be removed without disrupting the peptide host scaffold. Permanent porosity has been demonstrated by subsequent adsorption of other solvent molecules^[6] or various gases, including Xe,^[7] CO₂, CH₄, H₂,^[3a,b] Ar, N₂, and O₂.^[3c] In addition to absorption properties, these new bioorganic materials have been tested as nanovessels for chemical reactions.^[8]

Besides crystal structures of the 20 standard proteinogenic amino acids, the Cambridge Structural Database (CSD, version 5.36)^[9] contains crystal structures for a substantial number of other synthetic amino acids. By comparison, 100 out of 110 distinct dipeptides in the CSD, including all seven known members of the Val-Ala class,^[2a-c,5,6] have only proteinogenic residues. This restriction imposes clear limitations in terms of structural diversification. We therefore decided to explore an expansion of this family of hydrophobic dipeptides by incorporating the non-proteinogenic amino acids L-aminobutyric acid (Abu, ethyl side chain) and L-2-aminopentanoic acid (L-norvaline, Nva, *n*-propyl side chain). Although not derived from natural sources, both are bioactive: physiological testing has shown that Nva is an arginase inhibitor and regulates nitric oxide (NO) production in mammals and acts as an anti-inflammatory agent, while Abu is an active precursor used in both anticonvulsant drug molecules and antituberculous drugs.^[10] Abu and Nva residues are thus well suited as building blocks for the construction of biomaterials with potential use as drug carriers.

Herein, we present the synthesis and characterization of ten novel dipeptide structures **1–10** (Figure 1). They were crystallized in permanently porous architectures forming parallel and independent chiral channels with distinct diameters and helicities. Structure determination was provided by single-crystal X-ray diffraction analysis, while the accessibility



R = -CH₃: Abu-Abu (**1**), Abu-Nva (**2**), Abu-Val (**3**), Abu-Leu (**4**), Abu-Ile (**5**)
R = -C₂H₅: Nva-Abu (**6**), Nva-Nva (**7**), Nva-Val (**8**), Nva-Leu (**9**), Nva-Ile (**10**)

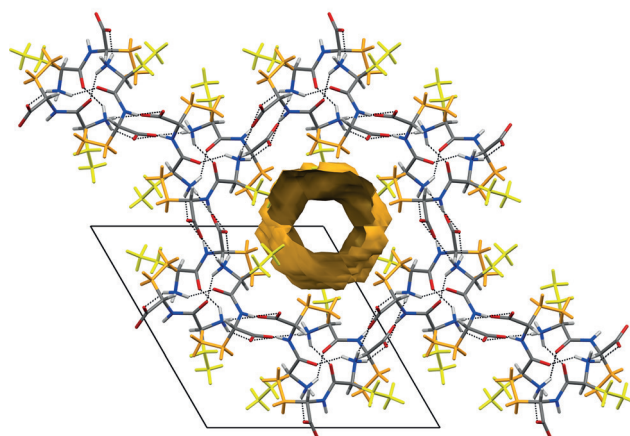


Figure 1. Chemical structures of the ten dipeptides (above) and the crystal structure of Abu-Abu **1** (below), with N- and C-terminal side chains colored yellow and orange, respectively.

[*] Dr. V. N. Yadav, Prof. T. Bonge-Hansen, Dr. M. Hennum, Prof. C. H. Görbitz

Department of Chemistry, University of Oslo
P.O.Box 1033 Blindern, 0315 Oslo (Norway)
E-mail: c.h.gorbitz@kjemi.uio.no

Prof. A. Comotti, Prof. P. Sozzani, Dr. S. Bracco
Department of Materials Science, University of Milano Bicocca
Via R. Cozzi 55, 20125 Milano (Italy)
E-mail: comotti@mater.unimib.it

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.201507321>.

of the matrix to CO₂ was proven by adsorption isotherms and by direct 1D and 2D MAS NMR detection.

The synthesis of the ten dipeptides (**1–10**) is reported in the Supporting Information. Single crystals were obtained by gel crystallization vapor diffusion techniques (acetonitrile vapors). XRD data collection at 105 K was followed by structure solution in space group *P*6₁ with *Z* = 6 (*Z'* = 1). The structures of **1–10**, exemplified by Abu-Abu (**1**) in Figure 1, 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 form connections to three neighboring double helices, thereby generating a robust supramolecular network. Abu-Leu (**4**) and Nva-Leu (**9**) are somewhat unexpected members of this family; Ala-Leu was obtained as a nonporous hemihydrate,^[11] and we had previously postulated that dipeptides containing Leu residues are incompatible with Val-Ala class *P*6₁ symmetry owing to steric conflict.^[2a] We now note that the assumed short intermolecular H⋯H distances can be alleviated by small changes in the main-chain and side-chain torsion angles.

The crystal channels (Figure 2) exhibit the shape of right-handed helices, with their grooves shaped by the terminal methyl groups of the peptide side chains. Abu-Abu (**1**) forms pores with a diameter of about 5.1 Å and has a void volume of 307 Å³ (17.4 % of the unit-cell volume), which is comparable to or higher than that of the channels of Val-Ala^[5] and Ala-Val.^[6]

The 3.3 Å pore size of Abu-Ile (**5**) is, surprisingly, smaller than the 3.7 Å of Val-Ile.^[2c] This observation may seem counterintuitive, since a Val residue contains an additional methyl group, but it reflects the substantial increase in unit-cell volume, from 1840.5(6) Å³ for **5** to 1940.8(4) Å³ for Val-Ile. 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**, thus resulting in a substantial reduction in channel diameters. Nva-Ile (**10**) shows the smallest cross-section of all of the dipeptides: 2.3 Å (the pore size is so small that the channels are hardly explored by any atom or molecule). Overall, there is an inverse correlation between side-chain bulkiness and the channel diameter, but with substantial variations depending on the specific residues involved (Figure 2). The crystal packing arrangements of peptides **1–10** have remarkably low density: among the compounds with formula C₈H₁₆N₂O₃, Abu-Abu (**1**) displays a crystal density as low as 1.06 g cm⁻³, which is comparable to the benchmark of the family L-Val-L-Ala (1.04 g cm⁻³ at 120 K).^[5] Furthermore, no guests occluding the channels were found by either ¹H or ¹³C fast-MAS NMR, thus demonstrating that the channels are empty.

The regular dipeptides of the Val-Ala class incorporate Val and Ile residues with branched side chains of limited conformational freedom. The more unrestricted ethyl and propyl side chains of Abu and Nva suggest that a diversity of side-chain conformations may be adopted, resulting in a soft aliphatic layer lining the channel walls. This feature was addressed by a comprehensive conformational analysis of all

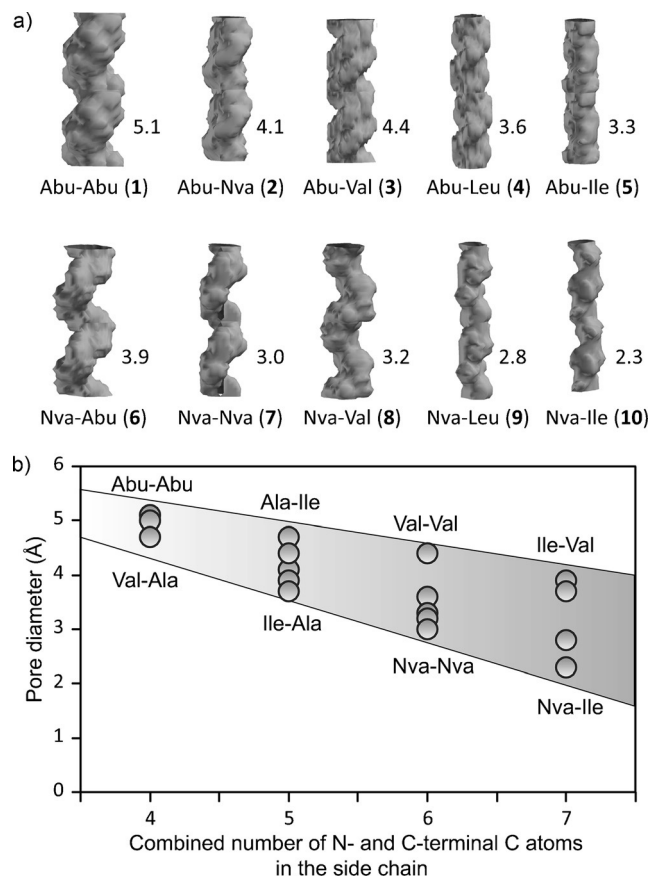


Figure 2. a) Channel-like pore volumes of the **1–10** crystals as explored by a sphere of 1.2 Å radius (1.0 Å for **10**) and 0.5 Å grid spacing. To the right of the channels, the corresponding cross-sections are reported (Å). b) Correlation between side-chain bulkiness and pore diameter for the seventeen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

17 dipeptides prepared so far, as well as eight potential, as yet uninvestigated, members of the Val-Ala class. The results of this investigation (see the Supporting Information) confirm that in the *P*6₁ space group, five out of the seven previous members of the Val-Ala class, including Val-Ala itself, are conformationally locked with respect to side-chain orientations. By contrast, Abu-Abu (**1**) and Nva-Abu (**6**) can explore five combinations of N- and C-terminal side-chain orientations. The channel shapes are consequently not fixed as in Figure 2a; the absorption of large guests may trigger conversion to other conformations. In this manner Abu-Abu channels may reach diameters close to 6 Å.

A special feature of peptides **6–10** is that the side chains of the N-terminal Nva residues cannot all adopt the preferred conformations with $\chi_1^2 = \text{trans}$,^[1c] since this would lead to prohibitively short intermolecular contacts at the center of the channels. The side chains are instead systematically disordered over alternating *trans* and *gauche* arrangements, thus resulting in local three-fold screw symmetry (Figure 3a). For calculations of void volumes, these structures were also refined (to higher *R*-factors) in the trigonal space group *P*3₁ (with *Z'* = 2).

¹³C Solid-state MAS NMR spectroscopy is very sensitive to conformations and motional behavior of alkyl chains, and

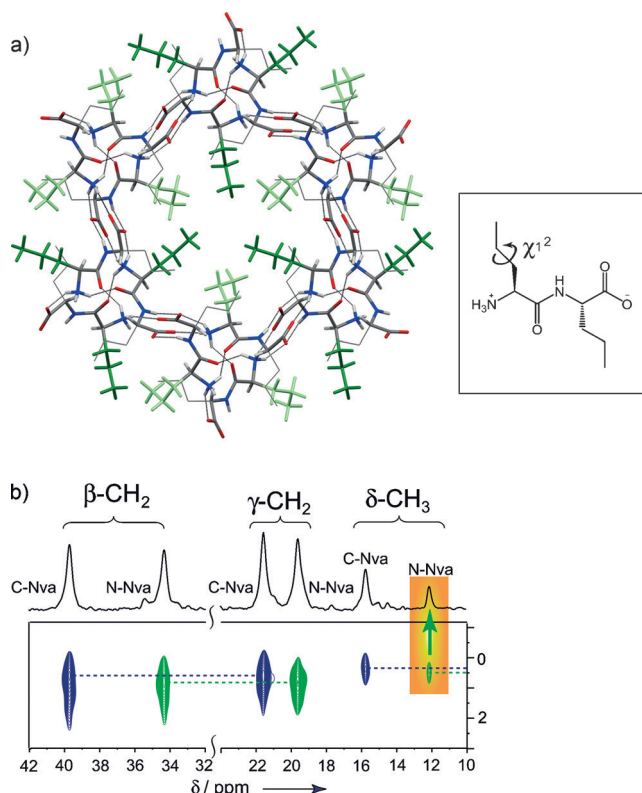


Figure 3. a) Crystal structure of Nva-Nva (**7**), showing alternating *trans* (dark green) and *gauche* (light green) conformations for χ_1^2 (defined in the insert) of the N-terminal side chains (C-terminal side chains appear in wireframe representation). b) 2D ^1H - ^{13}C NMR spectrum of Nva-Nva (**7**): expansion of the aliphatic region. The signal of the methyl group of the N-terminal Nva residue is highlighted in orange.

provides insight into the arrangement and mobility of the dipeptide side chains in the crystals. In the 2D ^1H - ^{13}C HETCOR MAS NMR spectra of Nva-Nva, Nva-Val, and Nva-Ile, we observed signals correlating to the pendant groups in the aliphatic region, in which the methyl groups of the N-terminal Nva residue resonate at $\delta_{\text{C}} = 12.5$, 12.3 and 13.0 ppm, respectively (Figure 3b). Such resonances are typical of *n*-alkane chain-end methyl groups experiencing the effect of *gauche* conformations of the vicinal $-\text{CH}_2-\text{CH}_2-$ bond (γ -*gauche* effect).^[12] Since the *trans*-to-*gauche* conversion accounts for a change of -5 ppm in hydrocarbons, the observed CH_3 upfield shifts of about 3 ppm with respect to the expected *trans* conformation resonance indicates that at least a 0.5 fraction of *gauche* conformation is explored, which is consistent with the 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, thus indicating an efficient relaxation mechanism of about 10^8 Hz. Consequently, in the family of dipeptides with N-terminal Nva residues, fast dynamic exchange between conformations occurs, which represents an intriguing case of fast dynamics in molecular crystals.^[13] The dynamic behavior is attained unconventionally through the generation of a channel-like free space, which promotes the mobility of the moieties that protrude towards the pores.

The empty space can be exploited for accommodating guests, which can diffuse in provided that 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 dipeptides was demonstrated through adsorption isotherms of gases. The isotherms for CO_2 and CH_4 with a few representative Abu- and Nva-containing dipeptides at 195 K and 1 bar are shown in Figure 4.

The CO_2 adsorption isotherms for Abu-Abu, Abu-Nva, Abu-Val, and Nva-Val show type I Langmuir behavior that is typical of microporous systems, and maximum absorption values (Q_{max}) of 62, 50, 41, 38 $\text{cm}^3(\text{STP})/\text{g}$, respectively. The CO_2 isotherms for dipeptides Nva-Nva and Nva-Leu follow a parallel pattern with lower sorption capacities. The maximum CO_2 absorption values in the series mirror the channel cross-sections, as determined from the crystal structures. The high capacity of Abu-Abu, equal to three CO_2 moles per unit cell, is consistent with its largest channel cross section of 5.1 Å and volume available (307 Å³/unit cell from the crystal structure and 297 Å³/unit cell from CO_2 adsorption). Moreover, CH_4 is adsorbed by Abu-Abu, reaching a Q_{max} of 38 cm^3g^{-1} , which corresponds to an empty space of 302 Å³/unit cell. The Langmuir curves of both gases are consistent with the ratio of gas volatility ($K_{\text{CO}_2}/K_{\text{CH}_4} \approx 4.8$) and their density in the liquid phase at 195 K. By contrast, Nva-Val exhibits an extremely low amount of adsorbed CH_4 , and the CO_2/CH_4 selectivity of 50 at 1 bar is due to its prohibitively small cross-section of 3.2 Å with respect to the diameter of CH_4 (4.4 Å). From variable temperature CO_2 isotherms, the

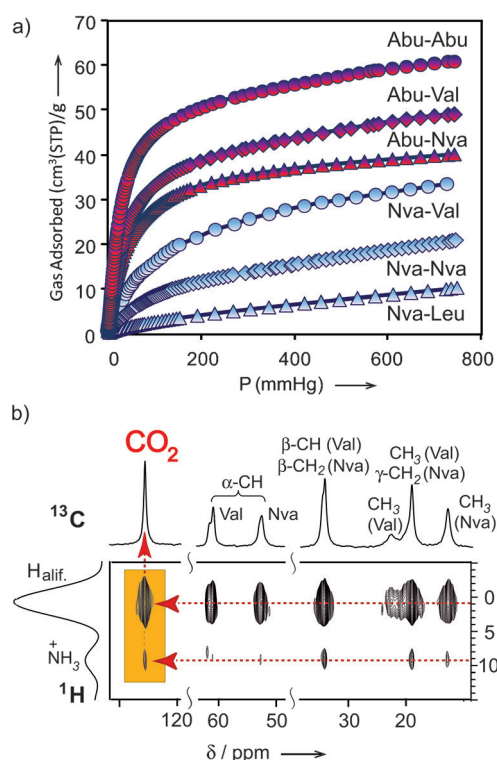


Figure 4. a) CO_2 adsorption isotherms for dipeptides Abu-Abu, Abu-Val, Abu-Nva, Nva-Val, Nva-Nva, and Nva-Leu at 195 K and up to 1 bar. Abu- and Nva-containing dipeptide series are indicated by red and blue labels, respectively. b) 2D ^1H - ^{13}C MAS NMR of Nva-Val loaded with ^{13}C -enriched CO_2 as recorded at 240 K. The cross-peaks between the host hydrogens and the CO_2 carbon nuclei are highlighted in orange.

isosteric heat of adsorption was measured to be 20 kJ mol⁻¹, thus revealing the formation of weak interactions between CO₂ and the aliphatic groups that line the channel walls.

Direct observation of CO₂ molecules inside the channels was provided by 1D and 2D ¹H-¹³C MAS NMR experiments with cross-polarization from hydrogen to carbon nuclei that reside at close distances for a residence time long enough to allow magnetization transfer (in the order of milliseconds).^[14] Indeed, when recorded at 240 K, the ¹³C CP MAS NMR spectrum of Nva-Val loaded with ¹³C-enriched CO₂ exhibits an intense signal at $\delta_c = 125.6$ ppm as a result of CO₂ adsorbed in the channels (Figure 4b). Since CO₂ does not possess hydrogen atoms, the sole source of magnetization is that conveyed from host hydrogen atoms to CO₂ carbon atoms in the surrounding space. Moreover, 2D ¹H-¹³C HETCOR experiments with Nva-Val/CO₂ show cross-signals, thus demonstrating close contacts between the aliphatic and ammonium hydrogen atoms of the host with CO₂ carbon atoms. Such experiments unequivocally prove close contact between the adsorbed gas and the porous solid at distances shorter than a few Å.

In conclusion, the use of non-proteinogenic amino acid residues allowed us to generate ten new crystal structures of hydrophobic dipeptides that show permanent porosity.^[15] The diversification of the amino acid building blocks substantially enriches the Val-Ala class and allows fine-tuning of the channel cross-section by systematically changing of the side chains. Adsorption isotherms at variable temperature demonstrate the availability of the crystalline channels to diffusing gases, showing preferred CO₂ uptake over CH₄ for Nva-Val. The present achievements pave the way for searches for yet other porous dipeptide materials constructed from even more unusual non-proteinogenic amino acids, such as fluoroalanine. Since the amino acids adopted here are non-hazardous, they could be used in medical and biological applications.

Acknowledgements

A.C. would like to thank PRIN 2011 and Cariplo Foundation for financial support.

Keywords: crystal engineering · bioorganic chemistry · microporous materials · peptides · X-ray diffraction

How to cite: *Angew. Chem. Int. Ed.* **2015**, *54*, 15684–15688
Angew. Chem. **2015**, *127*, 15910–15914

- [1] a) A. Aggeli, N. Boden, S. Zhang, in *Self-Assembling Peptide Systems in Biology, Medicine and Engineering*, Kluwer Academic Publishers, Dordrecht, **2001**; b) H. Hosseinkhani, P.-D. Hong, D. S. Yu, *Chem. Rev.* **2013**, *113*, 4837–4861; c) P. G. Vasudev, S. Chatterjee, N. Shamala, P. Balaran, *Chem. Rev.* **2011**, *111*, 657–687; d) X. Yan, P. Zhu, J. Li, *Chem. Soc. Rev.* **2010**, *39*, 1877–1890; e) M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee, N. Khazanovich, *Nature* **1993**, *366*, 324–327; f) J. B. Matson, R. H. Zha, S. I. Stupp, *Curr. Opin. Solid State Mater. Sci.* **2011**, *15*, 225–235; g) J. Rabone, Y.-F. Yue, S. Y. Chong, K. C. Stylianou, J. Bacsá, D. Bradshaw, G. R. Darling, N. G. Berry, Y. Z. Khimyak, A. Y. Ganin, P. Wiper, J. B. Claridge,

- M. J. Rosseinsky, *Science* **2010**, *329*, 1053–1057; h) C. Martí-Gastaldo, D. Antypov, J. E. Warren, M. E. Briggs, P. A. Chater, P. V. Wiper, G. J. Miller, Y. Z. Khimyak, G. R. Darling, N. G. Berry, M. J. Rosseinsky, *Nat. Chem.* **2014**, *6*, 343–351; i) C. Martí-Gastaldo, J. E. Warren, K. C. Stylianou, N. L. O. Flack, M. J. Rosseinsky, *Angew. Chem. Int. Ed.* **2012**, *51*, 11044–11048; *Angew. Chem.* **2012**, *124*, 11206–11210.
- [2] a) C. H. Görbitz, *Chem. Eur. J.* **2007**, *13*, 1022–1031; b) C. H. Görbitz, *Chem. Eur. J.* **2001**, *7*, 5153–5159; c) C. H. Görbitz, *New J. Chem.* **2003**, *27*, 1789–1793; d) R. Afonso, A. Mendes, L. Gales, *J. Mater. Chem.* **2012**, *22*, 1709–1723; e) I. W. Hamley, *Angew. Chem. Int. Ed.* **2014**, *53*, 6866–6881; *Angew. Chem.* **2014**, *126*, 6984–7000; f) S. Scanlon, A. Aggeli, *Nano Today* **2008**, *3*, 22–30; g) C. Valéry, F. Artzner, M. Paternostre, *Soft Matter* **2011**, *7*, 9583–9594.
- [3] a) A. Comotti, S. Bracco, G. Distefano, P. Sozzani, *Chem. Commun.* **2009**, 284–286; b) A. Comotti, A. Fraccarollo, S. Bracco, M. Beretta, G. Distefano, M. Cossi, L. Marchese, C. Riccardi, P. Sozzani, *CrystEngComm* **2013**, *15*, 1503–1507; c) R. V. Afonso, J. Durão, A. Mendes, A. M. Damas, L. Gales, *Angew. Chem. Int. Ed.* **2010**, *49*, 3034–3036; *Angew. Chem.* **2010**, *122*, 3098–3100.
- [4] S. Adiga, L. Curtiss, J. Elam, M. Pellin, C.-C. Shih, C.-M. Shih, S.-J. Lin, Y.-Y. Su, S. Gittard, J. Zhang, R. Narayan, *JOM* **2008**, *60*, 26–32.
- [5] C. H. Görbitz, E. Gundersen, *Acta Crystallogr. Sect. C* **1996**, *52*, 1764–1767.
- [6] C. H. Görbitz, *Acta Crystallogr. Sect. B* **2002**, *58*, 849–854.
- [7] a) D. V. Soldatov, I. L. Moudrakovski, J. A. Ripmeester, *Angew. Chem. Int. Ed.* **2004**, *43*, 6308–6311; *Angew. Chem.* **2004**, *116*, 6468–6471; b) I. Moudrakovski, D. V. Soldatov, J. A. Ripmeester, D. N. Sears, C. J. Jameson, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 17924–17929; c) D. V. Soldatov, I. L. Moudrakovski, E. V. Grachev, J. A. Ripmeester, *J. Am. Chem. Soc.* **2006**, *128*, 6737–6744.
- [8] G. Distefano, A. Comotti, S. Bracco, M. Beretta, P. Sozzani, *Angew. Chem. Int. Ed.* **2012**, *51*, 9258–9262; *Angew. Chem.* **2012**, *124*, 9392–9396.
- [9] C. R. Groom, F. H. Allen, *Angew. Chem. Int. Ed.* **2014**, *53*, 662–671; *Angew. Chem.* **2014**, *126*, 675–684.
- [10] a) X.-F. Ming, A. Rajapakse, J. Carvas, J. Ruffieux, Z. Yang, *BMC Cardiovasc. Disord.* **2009**, *9*, 1; b) M. A. Rogawski, *Br. J. Pharmacol.* **2009**, *154*, 1555–1557; c) P. E. Sanchez, L. Zhu, L. Verret, K. A. Vossel, A. G. Orr, J. R. Cirrito, N. Devidze, K. Ho, G.-Q. Yu, J. J. Palop, L. Mucke, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, E2895–E2903.
- [11] C. H. Görbitz, *Acta Crystallogr. Sect. C* **1999**, *55*, IUC9900149, cif-access.
- [12] a) M. Brustolon, A. Barbon, M. Bortolus, A. L. Maniero, P. Sozzani, A. Comotti, R. Simonutti, *J. Am. Chem. Soc.* **2004**, *126*, 15512–15519; b) A. Comotti, R. Simonutti, G. Catel, P. Sozzani, *Chem. Mater.* **1999**, *11*, 1476–1483.
- [13] a) C. S. Vogelsberg, M. A. Garcia-Garibay, *Chem. Soc. Rev.* **2012**, *41*, 1892–1910; b) A. Comotti, S. Bracco, A. Yamamoto, M. Beretta, T. Hirukawa, N. Tohnai, M. Miyata, P. Sozzani, *J. Am. Chem. Soc.* **2014**, *136*, 618–621.
- [14] P. Sozzani, S. Bracco, A. Comotti, L. Ferretti, R. Simonutti, *Angew. Chem. Int. Ed.* **2005**, *44*, 1816–1820; *Angew. Chem.* **2005**, *117*, 1850–1854.
- [15] CCDC 995070, 995071, 995072, 995073, 995074, 995075, 995076, 995077, 995078 and 995079 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre..

Received: August 6, 2015

Published online: September 28, 2015