DOI: 10.1002/cssc.201200098

Selective Oxidative Decarboxylation of Amino Acids to Produce Industrially Relevant Nitriles by Vanadium Chloroperoxidase

Andrada But,[a, b] Jérôme Le Nôtre,*[b] Elinor L. Scott,*[b] Ron Wever,[c] and Johan P. M. Sanders[b]

With growing ecological concerns, fluctuating oil prices and security of supply, there have been many developments towards the production of biofuels. More recently, the chemical industry has also started to expand towards biobased production routes for chemicals and materials. Alternative renewable resources such as carbohydrates, lignocellulosic biomass, glycerol, and fatty acids have become a main focus of research.[1]

In addition to conventional biobased feedstocks, research is also focusing on the transformation of protein-rich waste streams into bulk chemicals. Amino acids may be considered as an economically and energetically attractive raw material for nitrogen-containing chemicals. Their functionality reduces the need of energy-intensive processes to functionalize hydrocarbons with ammonia (or other reagents) and offers potential to circumvent process steps.[2] From the expected global rise in biofuels by 2020,[3a] and considering the European Union target of 10% of the total transportation market as biofuels by 2020,[3b] an additional 100 million metric tonnes of protein will be produced globally, corresponding to ca. 5 million tonnes of each amino acid. Not all amino acids are required for nutritional purposes. The isolation of non-essential amino acids, such as glutamic acid, could lead to their use without compromising feed or food value of the remaining composition. Glutamic acid is the most abundant amino acid in a number of biofuel rest streams, such as dried distillers grains and solubles (DDGS),[4] and could be considered as an interesting raw material for the production of industrial chemicals. Glutamic acid has been shown to be a versatile starting material for the synthesis of γ-aminobutyric acid (GABA),[5] *N*-methylpyrrolidone (NMP), *N*-vinylpyrrolidone (NVP),[6] succinonitrile,[7] and acrylonitrile.[8] Techno-economic and environmental assessments have shown the potential and points for further development in the use of glutamic acid as a platform chemical.[9] The main intermediate in the syntheses of succinonitrile[7] and acrylonitrile[8] from glutamic acid is 3-cyanopropanoic acid (**1**) (Figure 1). It is obtained by an oxidative decarboxylation reaction using sodium hypochlorite, and life-cycle assessment and comparison to petrochemical-based syntheses have shown that this step is detrimental.[9]

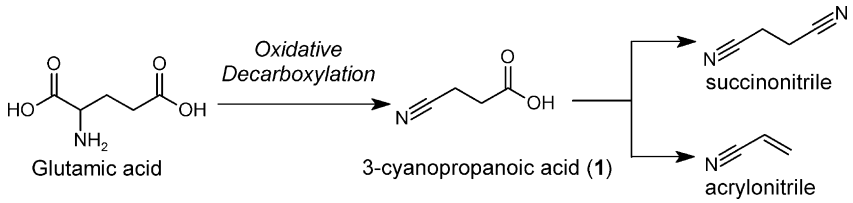
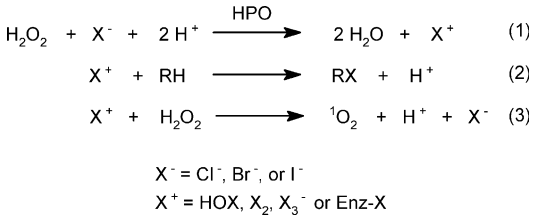


Figure 1. Biobased syntheses of succinonitrile and acrylonitrile from glutamic acid via 3-cyanopropanoic acid (**1**).[7–9]

Herein we report for the first time the conversion of glutamic acid to 3-cyanopropanoic acid (**1**) in high yield and selectivity using a vanadium chloroperoxidase enzyme (VCPO) and hydrogen peroxide. The reaction proceeds with a catalytic amount of sodium bromide and constitutes a general and more sustainable route to industrial nitriles from biomass due to the elimination of sodium hypochlorite.

A large number of halogenated compounds are produced in nature,[10] and the first halogenating enzyme, or haloperoxidase (HPO), was discovered in 1966 by Hager et al.[11] Two types of haloperoxidase co-exist in nature, depending on their prosthetic group which can be either an iron–heme complex or a vanadium oxide moiety. In the presence of hydrogen peroxide and a halogen source they produce a halogenating intermediate or "X^+" [Scheme 1, Equation (1)], which reacts with organic molecules to form the corresponding halogenated molecule



Scheme 1. General scheme for the halogenation reaction with haloperoxidase enzymes (HPOs).

[a] *A. But*
"Politehnica" University of Timisoara
Industrial Chemistry and Environmental Engineering Faculty
Piata Victoriei 2, 300006 Timisoara (Romania)

[b] *A. But, Dr. J. Le Nôtre, Dr. E. L. Scott, Prof. J. P. M. Sanders*
Chair of Biobased Commodity Chemicals
Wageningen University
PO Box 17, 6700AA Wageningen (The Netherlands)
Fax: (+31) 317-483011
E-mail: jerome.lenotre@wur.nl
elinor.scott@wur.nl

[c] *Prof. R. Wever*
Van't Hoff Institute for Molecular Sciences
University of Amsterdam
PO Box 94157, 1090 GD Amsterdam (The Netherlands)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cssc.201200098.

[Scheme 1, Equation (2)]. A reaction may also occur between X^+ and hydrogen peroxide which result in the formation of singlet oxygen [Scheme 1, Equation (3)].

In our study we used a vanadium chloroperoxidase (VCPO) obtained from the terrestrial fungus *Curvularia inaequalis* that has been fully characterized[12] and can be produced on a large scale.[13] This enzyme has a number of interesting properties, such as a high thermostability (90 °C), and can be stored at −20 °C over several years without activity loss. It also shows a very high resistance towards oxidants such as hydrogen peroxide (up to 100 mM) and singlet oxygen,[14] is stable in the presence of organic solvents,[15] and during conversions the enzyme remains fully stable for 25 000 turnovers. The K_m in bromide oxidation is unusually small (less than 10 μM), which means that the enzyme is fully functional at low bromide concentration.

Although both heme and vanadium peroxidases have been used in organic syntheses,[16] to halogenate or oxidize compounds there is only one report on the use of a heme containing bromoperoxidase in the transformation of amino acids to nitriles via an oxidative decarboxylation reaction.[17] The amino acids studied were converted into their corresponding nitriles. However due to a deamination reaction, aldehydes were obtained as side products and the ratio nitrile/aldehyde never exceeded 3:1. In the reaction mechanism a hypobromite ion is formed that reacts with the carboxylic function of the amino acid, inducing a decarboxylation. A second "Br^+" reacts with the primary amine and the nitrile function is formed. If the reaction is catalytic in bromide, two equivalents of oxidant are needed to reach complete conversion.

The bromoperoxidase activity of the VCPO enzyme used in this article has been well-studied, with turnover values ranging from 6 to 250 s^{-1} depending upon pH.[12a,18] First, phenylalanine (**2**) was chosen as a test substrate to be able to follow the reaction by HPLC analysis using UV detection and optimize the reaction conditions (Scheme 2). The reaction was performed at room temperature in citrate buffer at pH 5.6 containing 5 mM of phenylalanine (**2**), 2 mM of sodium bromide, and 18 nM of VCPO. Hydrogen peroxide (1.2 mM) was added every 15 min over a period of 5 h to maintain a low concentration and avoid singlet oxygen formation.[14] After screening the reaction at pH values from 5.0 to 6.2, a pH of 5.6 was selected at which the best conversion of phenylalanine (**2**) to phenylacetonitrile (**3**) was obtained with a minimum of degradation of the products (see Supporting Information).

Figure 2 shows that a complete conversion of phenylalanine (**2**) was observed after 4 h of reaction and phenylacetonitrile (**3**) was formed as the major product, together with phenylacetaldehyde (**4**) in a 3:1 ratio. Phenylacetonitrile production seemed to reach its maximum after 4 h, and the maximum concentration of phenylacetaldehyde (**4**) was obtained after 3 h. The results suggest that in the following two hours a degradation of phenylacetaldehyde (**4**) occurred, probably due to the presence of an excess of oxidant, where phenylacetonitrile (**3**) is not affected. Thus about 10% of the phenylalanine is lost. However, phenylacetonitrile (**3**) was produced in 70% yield.

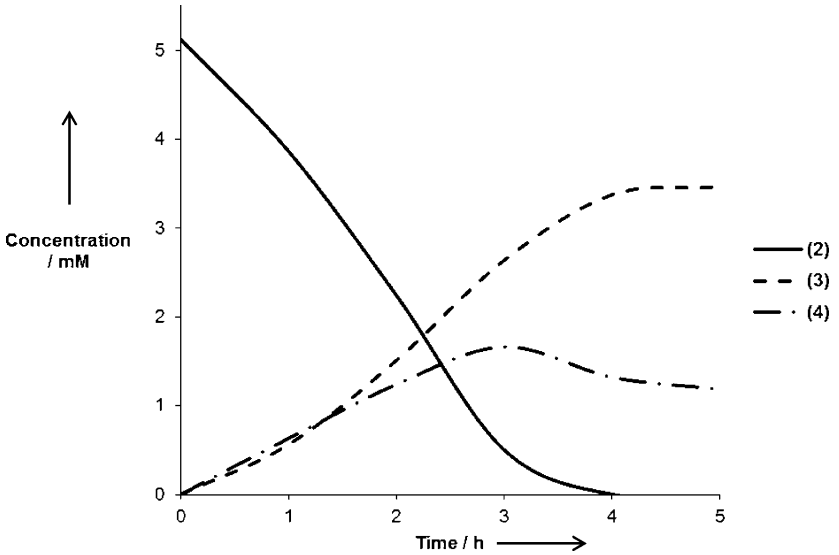
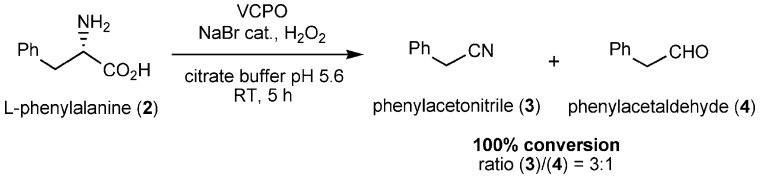


Figure 2. Phenylacetonitrile (**3**) and phenylacetaldehyde (**4**) formation from phenylalanine (**2**).

Because VCPO enzymes can also oxidize chloride, this halide (5 mM) was also tested in the reaction instead of bromide. However only 38% of phenylalanine (**2**) was converted after 5 h, probably due to the slower rate of oxidation of chloride by the enzyme.[12a] Furthermore, only phenylacetaldehyde (**4**) was formed and the selectivity of the reaction was completely inverted. This suggests that the mechanism of the reaction is dependent on the nature of the halide used. We did not investigate this further but the production of phenylacetaldehyde (**4**) from phenylalanine may be an interesting option because it is a valuable product in the fragrance industry.

Notably, phenylacetonitrile (**3**) and phenylacetaldehyde (**4**) are poorly miscible with water and therefore the samples were mixed with a solution of acetoni-



Scheme 2. Enzymatic oxidative decarboxylation of phenylalanine (**2**).

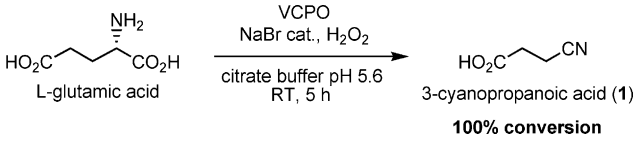
trile/methanol (1:1) to obtain homogeneous samples prior to analysis (see Supporting Information). This property is, however, an advantage in the isolation of the products. Phase separation or continuous extraction from an aqueous reaction mixture is in principle easily carried out. Indeed it was possible to continuously extract the aqueous reaction phase with different solvents, such as toluene, hexane, and ethyl acetate. Ethyl acetate gave the best results: it did not affect the rate of the reaction, slightly increased the selectivity towards phenylacetonitrile (**3**) (up to 83%), and especially the degradation of phenylacetaldehyde (**4**) did not occur.

The reaction conditions described above were then used in the conversion of glutamic acid at pH 5.6 (Scheme 3). The pH dependence was also studied and, as for phenylalanine (**2**), an optimal pH value of 5.6 to 5.8 was found (see Supporting Information).

As depicted in Figure 3, the rate of conversion of glutamic acid was similar to that of phenylalanine (**2**) and reached completion after 5 h. However, the selectivity was better, with only the nitrile product, 3-cyanopropanoic acid (**1**), formed and no product degradation. Thus the VCPO enzyme can transform glutamic acid into 3-cyanopropanoic acid (**1**) in complete conversion and high selectivity. Chloride was also tested instead of bromide, but the yield of 3-cyanopropanoic acid (**1**) reached only 50% after 5 h reaction, which is consistent with our previous conclusion on the choice of halide affecting reaction.

In our efforts to valorize protein-rich waste streams for the production of bulk chemicals, we often encounter problems in extracting amino acids from water and their separation prior to modification to bulk chemicals. In this respect the oxidative decarboxylation reaction by vanadium chloroperoxidase may be a very effective tool to perform, in a single step, the transformation of mixtures of amino acids into nitriles and the extraction of the hydrophobic products from the aqueous layer by phase separation. The water-insoluble nitriles could then be isolated by crystallization or distillation. To validate this idea we applied the haloperoxidase oxidative system to an equimolar mixture of phenylalanine (**2**) and glutamic acid (Figure 4).

After 4 h of reaction both amino acids were fully converted and the ratio of products obtained fit the individual reactions seen with each amino acid. Glutamic acid was transformed into 3-cyanopropanoic acid (**1**) without degradation; phenylacetonitrile (**3**) and phenylacetaldehyde (**4**) were produced from phenylalanine (**2**) with a 3:1 ratio and



Scheme 3. Enzymatic oxidative decarboxylation of glutamic acid into 3-cyanopropanoic acid (**1**).

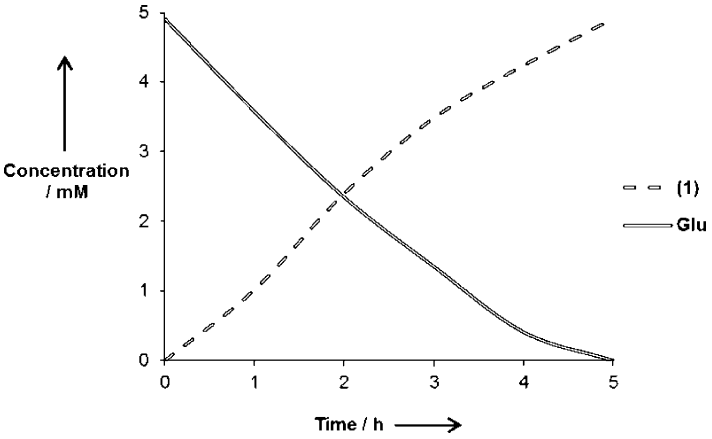


Figure 3. 3-Cyanopropanoic acid (**1**) formation from glutamic acid.

degradation of phenylacetaldehyde (**4**) was observed after 3 h of reaction.

This suggests that our oxidative decarboxylation system may be applied to a mixture of amino acids without any losses of reactivity and selectivity. Depending on the substrate range, it is conceivable that this enzymatic process could be applied to more complex mixtures of amino acids such as hydrolysate of protein-rich waste streams. Immobilizing the enzyme on a solid support[19,20] may allow recyclability and scale-up.

In conclusion, vanadium chloroperoxidase appears as an efficient and a selective tool to transform glutamic acid into 3-cya-

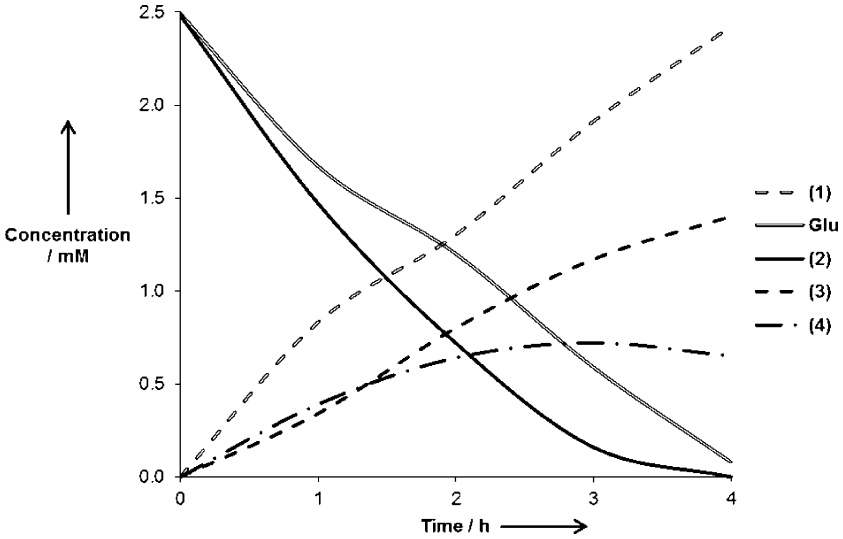


Figure 4. Products formation of the enzymatic oxidative decarboxylation performed on a mixture of phenylalanine (**2**) and glutamic acid.

nopropanoic acid (**1**). Compared to our previous studies, in which bleach was used to perform the reaction, this enzymatic process appears more sustainable and is likely to be more economically viable. Also during the process bromide is regenerated and only a low concentration is needed. Thus, it may be possible to develop a suitable biobased route to succinonitrile and acrylonitrile. More efforts are currently in progress in our laboratory to improve this transformation and to study its ecological and economic aspects. In addition the oxidative decarboxylation catalyzed via formation of HOBr by the stable vanadium chloroperoxidase may be used to convert a mixture of amino acids. For example the amino acids present in the hydrolysate of protein-rich waste streams could be transformed into nitriles of industrial interest. These are easily extracted from water and can be separated by conventional purification techniques, avoiding the difficulty of isolating individual amino acids from aqueous protein hydrolysate.

Experimental Section

Typical procedure for the VCPO-mediated oxidative decarboxylation of phenylalanine: In a 2 mL vial a solution containing 5 mM of phenylalanine, 2 mM of NaBr, 18 nM of VCPO, and 20 mM citrate buffer at the desired pH was stirred at 300 rpm at room temperature (21 °C). To this reaction mixture, small aliquots (2 μL×15) of H_2O_2 (24 mM) were added at intervals of 20 min. To prevent evaporation during the reaction the vials were closed with caps, the gas pressure was equilibrated when the covers were opened for the addition of hydrogen peroxide. The reaction was stopped by adding $Na_2S_2O_3$ to the mixture, 6 mL of acetonitrile/methanol (1:1, *v/v*) were added to homogenize the reaction mixture, and samples were taken for UHPLC analysis.

Acknowledgements

We are grateful to the Erasmus Programme for financial support (A.B.) and to Prof. Dr. Francisc Peter from the University of Timisoara. We also thank Susan Witte and Jana Kralova for their supports with HPLC and UHPLC analyses, and Maurice Franssen and Rokus Renirie for their helpful discussions.

Keywords: amino acids · biomass · enzyme catalysis · halides · oxidation

[1] a) A. Corma, S. Iborra, A. Velty, *Chem. Rev.* **2007**, *107*, 2411 – 2502; b) P. Gallezot, *Chem. Soc. Rev.* **2012**, *41*, 1538 – 1558; c) J. van Haveren, E. L. Scott, J. Sanders, *Biofuels Bioprod. Biorefin.* **2008**, *2*, 41 – 57; d) C. H. Christensen, J. Rass-Hansen, C. C. Marsden, E. Taarning, K. Egeblad, *ChemSusChem* **2008**, *1*, 283 – 289; e) J. J. Bozell, G. R. Petersen, *Green Chem.* **2010**, *12*, 539 – 554; f) J. Zakzeski, P. C. A. Bruijnincx, A. L. Jongerius, B. M. Weckhuysen, *Chem. Rev.* **2010**, *110*, 3552 – 3599.

[2] E. Scott, F. Peter, J. Sanders, *Appl. Microbiol. Biotechnol.* **2007**, *75*, 751 – 762.

[3] a) http://www.iea.org/press/pressdetail.asp?PRESS_REL_ID=411 and related report: http://www.iea.org/papers/2011/biofuels_roadmap.pdf; b) Directive 2009/28/EC of the European Parliament and The Council of April the 23rd 2009, *Official Journal of the European Union* **2009**, L140/16.

[4] a) T. M. Lammens, Ph.D. Thesis, Wageningen University, **2011**; b) V. Deike, J. D. Mol, *Raw Material Compendium*, Novus International, Brussels, **1996**; c) M. J. Spiehs, M. H. Whitney, G. C. Shurson, *J. Anim. Sci.* **2002**, *80*, 2639 – 2645; d) Y. Kim, N. S. Mosier, R. Hendrickson, T. Ezeji, H. Blaschek, B. Dien, M. Cotta, D. Dale, M. R. Ladisch, *Bioresour. Technol.* **2008**, *99*, 5165 – 5176.

[5] T. M. Lammens, D. De Biase, M. C. R. Franssen, E. L. Scott, J. P. M. Sanders, *Green Chem.* **2009**, *11*, 1562 – 1567.

[6] T. M. Lammens, M. C. R. Franssen, E. L. Scott, J. P. M. Sanders, *Green Chem.* **2010**, *12*, 1430 – 1436.

[7] T. M. Lammens, J. Le Nôtre, M. C. R. Franssen, E. L. Scott, J. P. M. Sanders, *ChemSusChem* **2011**, *4*, 785 – 791.

[8] J. Le Nôtre, E. L. Scott, M. C. R. Franssen, J. P. M. Sanders, *Green Chem.* **2011**, *13*, 807 – 809.

[9] a) T. M. Lammens, S. Gangarapu, M. C. R. Franssen, E. L. Scott, J. P. M. Sanders, *Biofuels Bioprod. Biorefin.* **2012**, *6*, 177 – 187; b) T. M. Lammens, J. Potting, J. P. M. Sanders, I. J. M. de Boer, *Environ. Sci. Technol.* **2011**, *45*, 8521 – 8528.

[10] G. W. Gribble, *Naturally Occurring Organohalogen Compounds—A Comprehensive Update*, Springer, **2010**.

[11] L. P. Hager, D. R. Morris, F. S. Brown, H. Eberwein, *J. Biol. Chem.* **1966**, *241*, 1769 – 1777.

[12] a) J. W. P. M. van Schijndel, E. G. M. Vollenbroek, E. G. M. R. Wever, *Biochim. Biophys. Acta Protein Struct. Mol. Enzymol.* **1993**, *1161*, 249 – 256; b) W. Hemrika, R. Wever, *Handbook of Metalloproteins* **2001**, 1416 – 1428; c) W. Hemrika, R. Renirie, S. Macedo-Ribeiro, A. Messerschmidt, R. Wever, *J. Biol. Chem.* **1999**, *274*, 23820 – 23827.

[13] P. Barnett, D. H. Hondmann, L. H. Simons, P. F. ter Steeg, R. Wever (Unilever), Int. Patent Appl. WO95/27046, **1995**.

[14] R. Renirie, C. Pierlot, J.-M. Aubry, A. F. Hartog, H. E. Schoemaker, P. L. Alsters, R. Wever, *Adv. Synth. Catal.* **2003**, *345*, 849 – 858.

[15] J. W. P. M. van Schijndel, P. Barnett, J. Roelse, E. G. M. Vollenbroek, R. Wever, *Eur. J. Biochem.* **1994**, *225*, 151 – 157.

[16] a) D. Wischang, O. Brücher, J. Hartung, *Coord. Chem. Rev.* **2011**, *255*, 2204 – 2217; b) A. Butler, *Coord. Chem. Rev.* **1999**, *187*, 17 – 35; c) D. Wischang, J. Hartung, *Tetrahedron* **2011**, *67*, 4048 – 4054; d) J. S. Martinez, G. L. Carroll, R. A. Tschirret-Guth, G. Altenhoff, R. D. Little, A. Butler, *J. Am. Chem. Soc.* **2001**, *123*, 3289 – 3294; e) J. N. Carter-Franklin, J. D. Parrish, R. A. Tschirret-Guth, R. D. Little, A. Butler, *J. Am. Chem. Soc.* **2003**, *125*, 3688 – 3689.

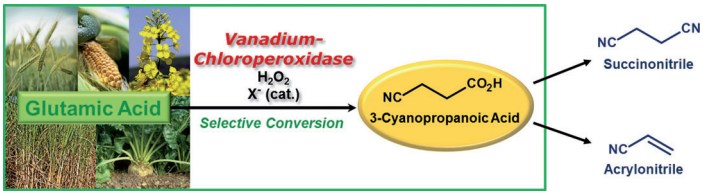
[17] M. Nieder, L. Hager, *Arch. Biochem. Biophys.* **1985**, *240*, 121 – 127.

[18] Z. Hasan, R. Renirie, R. Kerkman, H. J. Ruijssenaars, A. F. Hartog, R. Wever, *J. Biol. Chem.* **2006**, *281*, 9738 – 9744.

[19] M. Hartmann, C. Streb, *J. Porous Mater.* **2006**, *13*, 347 – 352.

[20] D. Wischang, J. Hartung, T. Hahn, R. Ulber, T. Stumpf, C. Fecher-Trost, *Green Chem.* **2011**, *13*, 102 – 108.

Received: February 8, 2012
Published online on ■■ ■■, 0000



Industrial nitriles from biomass: Vanadium-chloroperoxidase is successfully used to transform selectively glutamic acid into 3-cyanopropanoic acid, a key intermediate for the synthesis of bio-succinonitrile and bio-acrylonitrile, by using a catalytic amount of a halide salt. This clean oxidative decarboxylation can be applied to mixtures of amino acids obtained from plant waste streams, leading to easily separable nitriles.

A. But, J. Le Nôtre, E. L. Scott,* R. Wever, J. P. M. Sanders*

■■ – ■■

Selective Oxidative Decarboxylation of Amino Acids to Produce Industrially Relevant Nitriles by Vanadium Chloroperoxidase

