

Yves Robin



α -Amino acid *N*-Carboxy Anhydrides in pharmaceutical innovations: try them to capture new value

KEYWORDS: α -amino acid *N*-Carboxy Anhydrides, NCA, Urethane α -Amino acid *N*-Carboxy Anhydrides, UNCA, Ring Opening Polymerization, ROP, Peptide, Polypeptide, Polyamino acid, Amino acid coupling, Drug delivery, Active pharmaceutical ingredient.

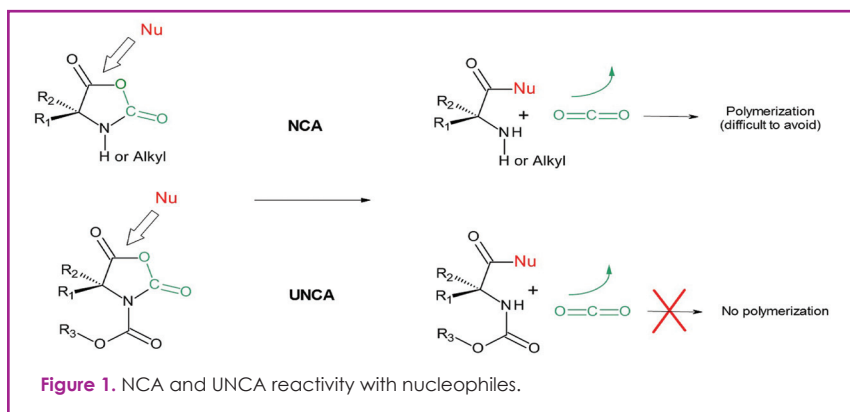
Abstract α -Amino acid *N*-Carboxy Anhydrides (NCA) have been discovered over a century ago (1). The first significant development and use of NCAs in the pharmaceutical industry started during the second half of the 20th century while the interest of chemists revealed three major areas for innovation: 1) their preparation 2) their use in ring opening polymerization and copolymerization (ROP) reactions for polyamino acid synthesis and 3) their use in coupling reactions for α -amino acid derivatives or peptide fragment synthesis.

An overview of NCA innovations are shown below in three main points: 1) The preparation of NCA building blocks from an industrial perspective. 2) The key role of NCAs in pharmaceutical applications and 3) The value of NCAs compared with other synthetic strategies of α -Amino acid couplings will be described.

WHAT ARE NCAs?

An NCA is a simple C-activated α -amino acid derivative that provides high electrophilic reactivity at the carbonyl group of the α -amino acid. Further protection of the amino function by urethane protecting group leads to α -amino acid the Urethane-*N*-Carboxy Anhydride (UNCA), an NCA subfamily.

NCAs are useful yet simple building blocks made of the α -amino acid or *N*-protected α -amino acid and one "carbon dioxide group" moiety. This "cyclic anhydride group" gives the NCA its high reactivity with various nucleophiles. The NCA reaction with nucleophiles is generally clean and leads to only carbon dioxide as the byproduct. This unique property makes these building blocks highly attractive when compared to other conventional strategies of α -amino acid activation which, quite often, generate large quantities of undesirable and hazardous side products which then need to be removed with tedious purification steps (Figure 1).



HOW ARE NCA AND UNCA SYNTHESIZED?

NCA synthesis

Since the first synthesis in 1922 (2), process chemists have made a lot of effort in order to develop efficient and scalable alternative synthesis. NCA can be prepared by three main strategies:

| Strategy | # of steps from α -Aminoacid | Reagents | By product - Waste |
|----------|-------------------------------------|---|---|
| 1 | 1 | Phosgene (or triphosgene) | HCl |
| 1bis | 1 | Diphenylcarbonate, or Carbonylimidazole | Phenol or imidazole |
| 2 | 2 | Thionylchloride + <i>N</i> -protecting reagents | HCl, SO ₂ , Alkyl halide |
| 3 | 2 | NO + carbamylation step | NO, N ₂ , H ₂ O ... |

Table 1. Comparison of the strategies for NCA synthesis

1. Reaction of phosgene with an α -amino acid (2)
2. Reaction of thionyl chloride with *N*-Boc or *N*-Cbz α -amino acid (3)
3. Nitrosation of an *N*-carbamoyl α -amino acid (4)

The first strategy using phosgene is very attractive because it involves a single step reaction and generates a low quantity of waste due to the nature of reagent (Table 1). Consequently, it has undergone a significant amount of development work over the years, either to improve the process based on phosgene or triphosgene (5-6) or to develop alternative processes using other phosgene substitutes such as carbonates (7) or other activated forms of carbon dioxide (8).

The generally preferred strategy of synthesis using phosgene has required substantial development in order to obtain a robust large-scale process for producing high purity NCAs. This is especially true for the use of NCAs in Ring Opening Polymerization (ROP) reactions (9). Indeed, when using phosgene or triphosgene, side reactions between the NCA and the generated HCl lead to an α -amino acid acyl chloride, hydrochloric acid as well as other impurities which gives a poor quality product with low yields when the process is scaled up (10-11).

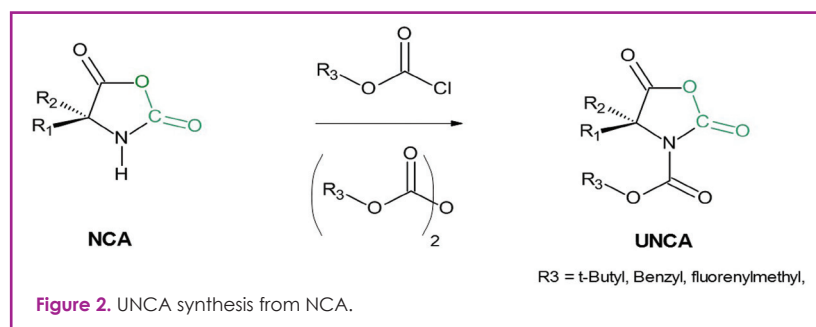
Several approaches have been developed to prevent these side reactions:

- Use of phosgene or triphosgene with a non-basic HCl scavenger (eg pinene and limonene) (5) or an improvement of the HCl removal (eg vacuum, stripping with nitrogen) (6);
- Use of non-chlorinated substitutes of phosgene (eg diphenylcarbonate) (7).

The last two strategies, which require two chemical steps, are less frequently encountered in the public information but they remain a back-up option on a case-by-case basis.

UNCA synthesis

UNCAs are prepared from NCAs via *N*-protection of the amine function using different protecting agents such as benzylchloroformate (CbzCl) for Cbz protection, di-*tert*-butyl dicarbonate (Boc₂O) or *tert*-butyl chloroformate for Boc protection and fluorenylmethylchloroformate (Fmoc-Cl) for Fmoc protection. The reactions are carried out in an anhydrous



solvent in the presence of an organic base and often at temperature of -20°C or below in order to prevent the competitive polymerization of the NCA (12) (Figure 2).

HOW DOES AN NCA COMPARE TO ALTERNATIVE STRATEGIES?

NCAs can be used in two major areas: synthesis of peptide polymer such as polyamino acids on one hand but also as an activated entity ready for use in peptide coupling reaction or in other nucleophile coupling reaction. Advantages and limitations of using NCAs in both uses is given in the tables below (Tables 2 and 4).

NCA for polyamino acid synthesis:

Table 2 is dedicated to the preparation of polyamino acid-based polymers by various methods. The ROP of NCAs is compared to other synthetic chemistry or biotechnology approaches (13):

The synthetic chemistry approach, and in particular the ROP of NCAs appears to be the most attractive option when targeting long polyamino acid chains. NCA monomers can be prepared from both natural and unnatural α -amino acids, including new side chain functional NCA monomers, which offer access to non-limited of functional polyamino acid (15). Developers of polymeric based nanomedicines are seeking well-defined and homogeneous polyamino acids architectures. The design of well-defined molecular weight average is key. Two mechanisms govern the polymer formation and consequently influence the molecular weight:

- The normal amine mechanism (NAM, nucleophilic attack of the carbonyl);
- The activated monomer mechanism (AMM, induced by nitrogen deprotonation).

NAM proceeds by an exclusive propagation pathway offering access to the controlled architectures. In contrast, AMM pathway relies on a deprotonated monomer reacting with and NCA which leads to an *N*-Aminoacyl-NCA intermediate A (Figure 3). The intermediate can then either react with an NCA monomer in the propagation pathway or react with another *N*-Aminoacyl-NCA leading to higher different molecular polymer. Thus, AMM pathway must be avoided for a successful preparation of high molecular weight and low polydispersity index polyamino acids by ROP.

Many ROP technics have been developed and can be compared as follow (Table 3).

UNCA for α -amino acid coupling synthesis

α -amino acid and peptide couplings have drawn a lot of attention since the middle of the 20th century. The peptide chemists are constantly looking for efficient synthesis technics. Is there a best strategy and an universal reagent to make all amide bond formation? The answer is clearly no (15). Several parameters have to be taken into account such as: solution or solid phase process, scale of production, excess of reagents, side chain functional groups, isolation and purification.

WHAT ARE NCA CHEMISTRY ACHIEVEMENTS TODAY?

Marketed API's synthesis

NCA and UNCA have proven to be very useful in synthesis of APIs. Three examples from the literature show the interest of using of NCAs or UNCAs in large scale API manufacturing process.

Synthesis of ganciclovir valinate (Valganciclovir) a prodrug of ganciclovir. The reaction of *N*-Benzyloxycarbonyl-L-Valine-*N*-Carboxyanhydride (Z-Val-NCA) with Ganciclovir carried out in smooth conditions without any coupling agents is claimed to be an attractive alternative to synthesis to other coupling techniques starting from *N*-Benzyloxycarbonyl-L-Valine (16) (Figure 4).

Synthesis of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid *tert*-butylamide, a key intermediate of Saquinavir and Nelfinavir synthesis. An attractive process is claimed starting from 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid which is converted to the corresponding NCA by reaction with triphosgene and then reaction of the NCA with an excess of *tert*-butylamine (17) (Figure 5).

NCAs are proven to be useful monomers for peptide polymer synthesis. Glatiramer acetate is the first example of peptide copolymer drug (18). Glatiramer acetate is prepared by a random ring opening copolymerization of four natural α -amino acids

based NCAs, L-Alanine-NCA, ϵ -trifluoroacetyl-L-Lys-NCA, γ -Benzylglutamate-NCA and L-Tyrosine-NCA. The protected copolymer undergoes hydrobromic acid hydrolysis and purification. The Glatiramer acetate is then obtained by ion exchange.

Polymer therapeutics in clinical developments

A dozen of polypeptide based polymer therapeutics are currently in clinical trials (8). A selection of two advanced clinical development applications is given below:

| Family | Technics | Advantage * | Limitations* |
|---------------------|-------------------------------|---|--|
| Synthetic chemistry | Native Chemical Ligation | Absence of complex protecting groups | - Restricted to link peptide segment which, often based on Solid Phase Peptide Synthesis (SPPS) - Scale-up issues due to instability of the nhoiester moiety at the reaction pH - Expensive |
| | Solid Phase Peptide Synthesis | Access to complex peptide polymer composition including unnatural amino acids Access to well defined molecular weight polymers | - Limitations in polypeptide length and purity for long polypeptides - Expensive compared to ROP of NCAs |
| | ROP of NCA | Access to - polypeptides beyond nature's possibility (including unnatural amino acids) - wide range of molecular weight | - Requires high purity NCA and efficient catalyst system to control chain length and polydispersity index - Limitation to block or random polymers and copolymers |
| Biotechnology | Recombinant DNA Technics | Molecular weight, Sequence and stereochemistry defined on genetic template Access to correct conformation | - Effort for development of the gene and cell expression (cost and timelines) - Multi-domain proteins challenging to express - Low protein production due to low compatibility with bacteria - Incorporation of non-natural α -amino acids is not yet achieved |

*adapted from (14)

Table 2. Polypeptide-based polymer synthesis strategies.

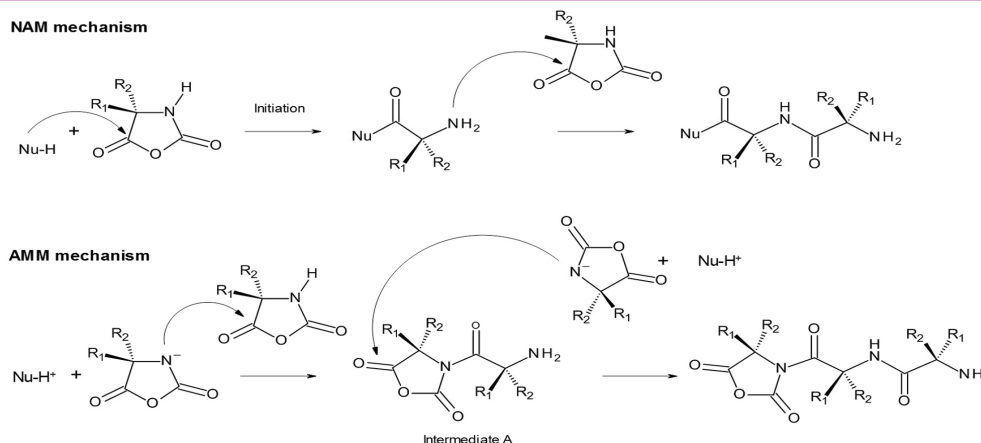


Figure 3. Ring opening polymerization mechanism.

| Polymerization Technics / catalyst | ROP Control (PDI and Mw) | Catalyst | Implementation | Common key success factor |
|------------------------------------|------------------------------|----------------------------------|----------------|---------------------------|
| Heavy metal | High | Metal complex, toxic issue | Easy | High purity monomers |
| High Vacuum technics | High | No catalyst no toxicity issue | Complex | |
| Primary amines and low temperature | Limited | Simple, no toxicity issue | Easy | |
| Hexamethyldisilazanes catalyst | Limited (and low reactivity) | Simple, no toxicity issue | Easy | |
| Amines hydrochlorides | Limited | Simple, no toxicity issue | Easy | |
| Amines tetrafluoroborates | High | Simple, no toxicity issue | Easy | |

Table 3. Comparison of ROP Technics - adapted from (8)

Otherwise, cost of the peptide active ingredient is critical in the drug development. The synthetic approach and the purification are key parameters to consider. Table 4 summarizes few methods based on activation strategies.

Beside the amide bond formation from NCA or UNCA, other reactions with various nucleophiles are not all disclosed here. Among them, preparation of ester can be advantageously achieved by the reaction of UNCA with an alcohol (16).

WHAT IS THE FUTURE OF NCAs?

API synthesis

Although NCAs have been successfully used in API synthesis as previously shown. They are not sufficiently known and thus not enough considered in manufacturing processes. The NCAs' advantages (no racemization, fast reactions, easy work-up and no by products) should be more considered by process chemists and even early enough by medicinal chemists. Nevertheless, recent patent applications over the last 5 years emphasize that NCA technology is chosen by chemists for both generic API synthesis and new chemical entities (Table 5).

The use of NCAs in API synthesis strategies is certainly promised to grow as research and process chemists consider this strategy based on the successful examples in the literature. The strength of this technology for industrial processes is demonstrated by its lower environmental and safety impact when compared to other coupling agents strategies. It also offers product purity profile advantage due to the reaction selectivity and the absence of any coupling reagent.

Polypeptide based polymers

The use of NCAs for the preparation of α -amino

acid based polymers has been known for decades. Most recently, innovations in drug delivery systems and active stream directly from progress in synthetic polypeptide-based polymer methodologies' access to offering more sophisticated architectures. For example, alternatively to the classical anhydrous medium ROP, an aqueous medium ROP process has been developed for grafted poly-L-Lysine dendrimers (21).

In addition, linear polymers for drug delivery technologies as well as well-defined and structured polymer architectures like dendrimers or block copolymers are developed for various applications including bacterial and virucidal applications (8). The most advanced

| Family | Technics | | Advantage ** | Limitation** |
|--------------------------|-----------------------|---|---|--|
| Non shelf stable reagent | Coupling reagents | Phosphoniums, uroniums, organophosphorous, organosulfur, others | - Wide range of reagents - High efficiency - Applicable to α -amino acid coupling and peptide coupling | - Coupling by-products - Potential hazardous reagents |
| | Non cyclic Anhydrides | Mixed carbonic anhydrides | - Applicable to α -amino acid - coupling and peptide coupling - Low Cost | - Require accurate optimization |
| Shelf stable reagent | Acid halides | Acid chlorides and Fluorides | - Simple halogen leaving group - High reactivity for hindered substrate - Low cost | - Racemization - Side reactions - Not applicable for peptide coupling - Side chain protection compatibility |
| | Active esters | Phenyl derivatives Hydroxamic derivatives | - Applicable to α -amino acid coupling and peptide coupling - α -amino acid esters can be easily purified | - Elimination of the leaving group of the ester (waste) |
| | Non cyclic Anhydrides | Symmetric anhydrides | - α -amino acid anhydrides can be easily purified | - Loss of half of the symmetric anhydride - Potential issue with removal of the unreacted moiety |
| | Cyclic anhydrides | UNCA | - High reactivity for hindered substrates - High selectivity - No racemization - Only Carbon dioxide as coupling by product - Little need of post reaction purification | - Not applicable to all α -amino acid coupling and peptide coupling due to a limited list of available UNCAs Mainly limited to solution phase coupling |

**selection of main items as a general trend

Table 4. Strategies of activation of the carboxylic acid of α -aminoacids.

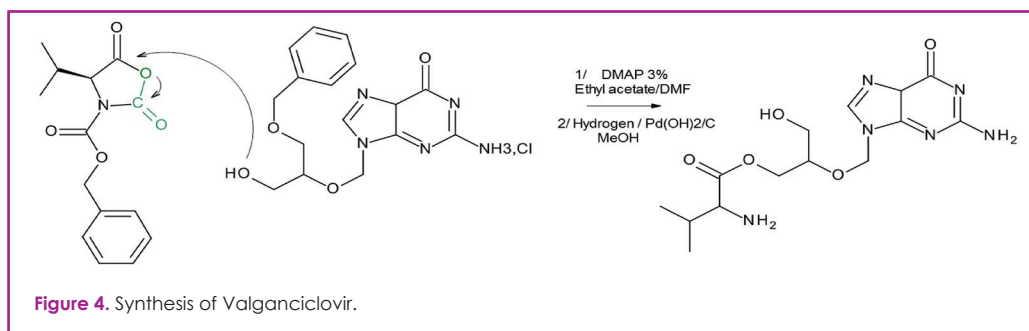


Figure 4. Synthesis of Valganciclovir.

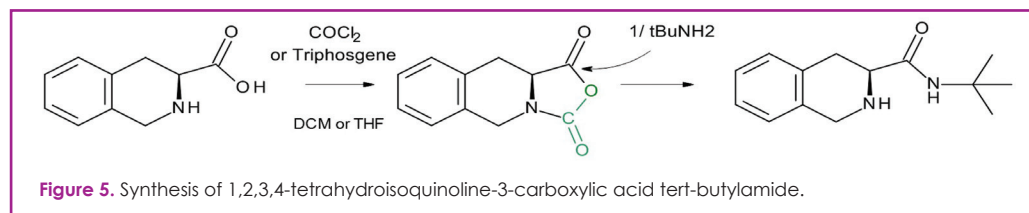


Figure 5. Synthesis of 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid tert-butylamide.

Polymer drug conjugate:

Opaxio™ (Cell therapeutics Inc.): This is a PGA-Paclitaxel (PTX) conjugate for the treatment of various cancers including ovarian, prostate or head and neck carcinomas (Phase III) (19). The polyglutamic skeleton is prepared by biotechnology.

Polymeric Micelles:

NK105 (NanoCarrier Co.-Nippon Kayaku Co): This is a mPEG-poly(aspartic acid) (PTX) micelle for the treatment of recurrent or meta-static breast cancer (Phase III) (20). The polyaspartic acid skeleton is prepared by ROP.

-20°C of two years in the worst case (24).

In any case NCAs can be warmed up to room temperature for hours in order to ease product handling during the downstream process.

CONCLUSION

NCAs are an established strategic building block family for ROP reactions offering access to peptide based therapeutic polymers. Ongoing research on both poly-aminoacid architectures and biological

activities associated with these synthetic polymers should provide successes in the field of polymer drugs or polypeptide carriers in drug delivery and targeting technologies. Expanding the range of available NCA monomers, including side chain functionalized α -aminoacids, offers post-polymerization modifications which broaden the perspectives of α -amino acid based polymer therapeutics (14).

UNCAs are also powerful building blocks in α -amino acid couplings and are often used in API and NCE synthesis. They are now an established and attractive alternative to traditional α -amino acid activation strategies for coupling reactions. The future development of industrial applications is now in the hands of medicinal chemists and more importantly process chemists who make the choice on the route of synthesis of NCEs and later on large scale APIs production. Both NCA applications require industrial production of NCAs. Current process knowledge allows to provide high quality NCAs from laboratory to large production scale. Although NCAs are highly reactive and sensitive to both moisture and temperature, suitable packaging and temperature controlled logistic solutions have been developed which make them available at industrial scale and allow shipment all over the world (25).

REFERENCES

- H. Leuchs, Ber. Dtsch. Chem. Ges., 39, 857-861 (1906)
- P. L. Fuchs, Chem. Ber., 1922 (55), 2943.
A. C. Farthing, A. C. J. Chem. Soc., 1317 (1950)
- H. Leuchs, Ber. Dtsch. Chem. Ges. 39, 857-861 (1906)
H. Leuchs, Ber. Dtsch. Chem. Ges., 40, 3235-3240 (1907)
- EP 1024133 and FR 2899583
- EP 1201659 B1
- EP1262480 / WO2006/047703
- US 2007/0015932
- US 2002/0188095
- Selection of reviews on ROP:
H. R. Kricheldorf, Angew. Chem. Int. Ed., 45, 5752-5784 (2006)
A. Duro-Castano, I. Conejos-Sánchez and M. J. Vicent, Polymers, 6, 515-551, 2014
H. Lu, J. Wang, Z. Song, L. Yin, Y. Zhang, H. Tang, C. Tu, Y. Lin and J. Cheng, Chem. Comm., 50, 139-155, 2014
N. M. B. Smeets, P. L. Van der Weide, J. Meuldijk, J. A. M. Vekemans, and L. A.

| Application | Use | Technology | Patent reference |
|---------------|--|---------------------------------|------------------------------|
| API synthesis | Synthesis of Ramipril | NCA coupling | WO2012/063252 |
| API synthesis | Synthesis of Sitagliptin from L-aspartic acid | NCA in Friedel & Craft reaction | US 2011-68713 |
| API synthesis | Synthesis of a Bortezomib intermediate | NCA coupling | CN102268029 |
| API synthesis | Preparation of Aspxocillin | NCA coupling | CN103232475 |
| NCE synthesis | Synthesis of quinazolines Jak kinase modulating | UNCA coupling | WO2010099379 WO2012030944 |
| NCE synthesis | Preparation of modified nicotinic compounds as B cell antigens and related methods | NCA coupling | WO 2012061717 |
| NCE synthesis | Preparation of heterocyclic urea derivatives as antibacterials | UNCA coupling | WO 2011024004 |
| NCE synthesis | Hydroxyalkyl benzyl pyrazoles, the treatment of hyperproliferative and angiogenic diseases | UNCA coupling | WO 2011141325 |

Table 5. Recent patent application using in NCA Technology.

example is VivaGel® (Starpharma) currently in clinical trial phase III development for the treatment and prevention of bacterial vaginosis and the prevention of sexually transmitted infections. This polymeric drug is based on a polylysine dendrimer (22). Another example is with A-Blocks™ (Amicrobe Inc.) cationic amphiphile α -amino acid based polymers which are under preclinical development as antibacterial for surgical hydrogels (23).

Numerous research and early stage projects foresee a promising future for poly aminoacid based polymers in pharmaceutical therapies (8,14).

WHAT ABOUT NCAs STABILITY?

NCAs and UNCAs are known to be highly reactive and they are deemed to be unstable. To the question: Is it possible to produce, store, transport and use NCAs at large scale? The answer is clearly yes. However, the stability of NCAs and UNCAs basically depends on their purity and storage conditions. Otherwise, the nature of the side chain moiety of the NCA influences the stability.

NCAs are highly reactive, they undergo polymerization and they are sensitive to nucleophiles and moisture. UNCAs are sensitive to nucleophiles, moisture and temperature but does not undergo polymerization like NCAs thanks to the urethane protecting group. In both cases, nitrogen atmosphere, moisture proof packaging and cold storage are proven to be efficient enough to prevent both hydrolysis and/or polymerization at industrial scale.

The following examples (Table 6) demonstrate a stability at

| NCA | CAS RN# | Shelf life at -20°C | Remark |
|--|-------------|---------------------|------------------|
| L-Alanine N-Carboxyanhydride | 2224-52-4 | 2 years | / |
| L- γ -Benzyl glutamate N-Carboxyanhydride | 3190-71-4 | 3 years | / |
| L- γ -Methyl glutamate N-Carboxyanhydride | 1499-55-4 | 2 years | / |
| L- ϵ -Trifluoroacetyl lysine N-Carboxyanhydride | 42267-27-6 | 5 years | / |
| L-Tyrosine- N-Carboxyanhydride | 3415-08-5 | 5 years | 9 months at 20°C |
| Cbz-L-Valine N-Carboxyanhydride | 158257-41-1 | 3 years | 2 months at 20°C |

Table 6. Stability data according to ICH guidelines.

- Hulshof, Org. Process. Res. Dev., 9(6), 757, 2005
11. Y. Robin, Production process of α -Amino acid N-Carboxyanhydrides: Batch process development, scale-up concerns and industrial solution Org. Process Res. Dev., Conference and Exhibition, Lisbon, 9th – 11th September 2009
 12. R. H. Kricheldorf, Makromol. Chem., 178, 905, 1977
D. W. Fuller et al., US 5028693
J. Am. Chem. Soc. 112, 7414, 1990
 13. A. Duro-Castano, I. Conejos-Sánchez and M. J. Vicent, Polymers, 6, 515-551, 2014
 14. El-Faham A, Albericio F. Peptide Coupling Reagents, More than a Letter Soup. Chem. Rev., 111, 6557-6602, 2011
 15. H. Lu, J. Wang, Z. Song, L. Yin, Y. Zhang, H. Tang, C. Tu, Y. Lin and J. Cheng, Chem. Comm., 50, 139-155, 2014
 16. US 6040446
 17. US6340760 & ISOICHEM's unpublished results
 18. Drug of the future; 20(2), 139-141, 1995
 19. Galic, V.L.; Wright, J.D.; Lewin, S.N.; Herzog, T.J. Paclitaxel poliglumex for ovarian cancer. Expert Opin. Investig. Drugs, 20, 813-821 2011
 20. www.nanocarrier.co.jp/en/research/pipeline/01.html
 21. PCT/FR2006/000952
 22. http://www.starpharma.com/vivagel
 23. WO2012027411 - http://www.amicrobe.com/scienceofablocks.html
 24. ISOICHEM stability study data according the ICH Guideline
 25. ISOICHEM : www.isochem.eu , and N-Carboxy anhydride brochure



OCTOBER 20-21 • Houston Marriott West Loop • Houston, TX

Process Safety and Operational Excellence

A "Process Safety Incident Warning Signs" workshop will be followed by presentations, panels and discussions from national and local authorities on process and occupational safety. Join us in celebrating the 100 year anniversary of ChemStewards® and to learn the latest trends and methodologies used in the industry today to create a safe and secure manufacturing environment.

Register now at

www.socma.com/safety/symposium

Official
Media Partner: **C&EN**

