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Polypeptoids are an emerging class of biomimetic polymers prepared by ring-opening polymerization (ROP) of N-alkylated-N-carboxyanhydride (NNCA) monomers. The N-alkylation provides a unique structural isomerism with peptides but its influence on NNCA reactivity is not yet fully understood. In this report, we provide a comprehensive study using 12 monomers to better rationalize the contribution of the steric hindrance and electronic effects of the N-alkyl group toward the synthesis of NNCA and their reactivity in ring-opening polymerization (ROP) reactions. We found that varying the alkyl group does not significantly influence the formation of NNCAs prepared by the Leuchs' method. In marked contrast, depending on the alkyl group, the efficiency of the NNCA polymerization initiated by allylamine showed that electron-donating groups enhanced the ROP kinetic rates through significant inductive effect and could counterbalance the negative influence of bulky groups during the propagation steps.

Introduction

Peptoid polymers, also called poly(N-substituted-glycines) or polypeptoids are N-alkylated analogs of synthetic polypeptide polymers: they are polypeptides with an N-substituted amide bond.1,2 The physicochemical properties of polypeptoids can be tailored by the N-alkylation, enabling control over 1) the hydrophilic to lipophilic balance,3 2) the charge characteristics,4,5 3) the backbone conformation,6 4) the solubility,7 5) the thermal and crystallization properties.8,9 The most direct way to prepare polypeptoids is to polymerize N-alkylated-N-carboxyanhydride monomers (NNCA). Historically, the Leuchs method made use of thionyl chloride ($SOCl_2$)10 or other halogenated electrophilic reagents such as PCl_3 and PBr_3.11,12 Adaptation of the Leuchs method later involved a mixture of acetyl chloride and acetic anhydride,13 or the use of alternative reagents.14 More recently, NNCAs production was also achieved using phosgene or its derivatives, a strategy called the Fuchs Farthing method.7 The advantage of this approach relative to the Leuchs method is that there are fewer chlorine acid traces in the reaction medium that can retard polymerization, even though recent improvements in purification processes tend to mitigate this advantage.15–17

NNCA polymerization is a controlled process when initiated with nucleophiles such as amines (primary or secondary),7 strong bases such as LiHMDS18 or N-heterocyclic carbenes.12 Recent developments includes the use of organocatalysis to speed up the ROP kinetics such as 1,3-bis[3,5-bis(trifluoromethyl)phenyl]urea,19 7-methyl-1,5,7-triazabicyclo[4.4.0]dec- 5-ene (MTBD),20 and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).21 These different methods all result in a living polymerization process: the addition of more NNCA monomers leads to a stoichiometric increase in the number average length of the polymer chains,22 and this enables the preparation of both random23–25 and block copolymers.22,26–28 The most studied polypeptoid is obtained by polymerizing N-methyl-glycine-N-carboxyanhydride, also called sarcosine NCA, (Sar-NCA). Its polymerization proceeds in a controlled manner without chain transfer or termination events, producing polysarcosines with narrow molar mass distributions (ĐM = 1.1-1.3).22 The same behavior has been observed with other NNCAs bearing a range of N-alkyl side chains.7,29 The chemical nature of the N-alkyl group strongly influences the ROP kinetics, which are much

slower than those of Sar-NCA for most NNCAs.19 Previous studies involved NNCAs with varying aliphatic chains 1,7,30–33 or with heteroatom-containing side chains N-(3-tert-butoxy-3-oxopropyl) NNCA,5 N-2-methoxyethyl-NCA (MeOEt NCA) and N-2-(2'-methoxyethoxy)ethylglycine derived NCA (Me(OEt)2 NCA).34 For most of these NCAs, the polymerization rate depended primarily on steric hindrance,35 but the electron-withdrawing effect of substituent could also inhibit the ROP.36

In this work, we present a study based on 12 NCA monomers for which synthesis and polymerization kinetics were evaluated by infrared spectroscopy and molecular modelling. The obtained polymers were also fully characterized by a combination of NMR, SEC and MALDI-TOF analyses. The objective of this study was to better understand the influence of N-alkylation on the efficiency of ring closure during the formation of NNCA and on ring opening, during their polymerization. While for the formation of NNCAs by the Leuchs method, N-alkylation did not strongly influence the cyclization kinetics, our study revealed that steric hindrance can be significantly counterbalanced by positive inductive electronic effects activating the propagation steps of the ROP.

The main text of the article should appear here with headings as appropriate.

Experimental

Materials and methods

Materials. All chemicals and solvents were purchased from Sigma Aldrich, Fluorochem, Acros, TCI, Strem and, unless otherwise described, were used without further purification. Dimethylformamide (DMF) and tetrahydrofuran (THF) were obtained from a solvent system purifier (PureSolv, Innovative Technology), kept under argon atmosphere and freshly used. MilliQ water was obtained from a system purifier (Purelab Prima, ELGA). The monomers synthesized were stored at 4 °C under argon atmosphere and weighed in a glove box (Jacomex GP13 No. 2675) at the Laboratoire de Chimie des Polymères Organique (LCPO, Pessac, France).

Methods. The IR spectra were recorded using an FTIR spectrometer (Vertex 70, Bruker), and the samples were measured with an ATR (GladiATR, Pike Technologies) from Fisher technologies performing 32 scans at the LCPO (Bordeaux, France). The raw data were obtained with Opus7.5 software and processed using Originlab 2016 software. The NMR spectra were recorded using a Bruker Avance III 400 spectrometer (LCPO, Bordeaux, France). The spectra were obtained at 295 K and data was analyzed using Mestrenova 14.1.2 software. The chemical shifts of the signals are given in ppm. The spectra obtained were calibrated using the residual solvent signals ($CHCl_3$ 7.26 ppm, H_2O 4.79 ppm, DMSO-d6 2.50 ppm). The signals were categorized as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) and broad (br). Polymer molar masses were determined by Size Exclusion Chromatography (SEC) using: a) Dimethylformamide (DMF + lithium bromide LiBr 1g/L) as the eluent (LCPO, Bordeaux, France). Measurements in DMF were performed on an Ultimate 3000 system from Thermoscientific equipped with diode array detector DAD. The system also included a multi-angle light scattering detector MALS and differential refractive index detector dRI from Wyatt technology. Polymers were separated on two Shodex Asahipack gel columns GF310 and GF510 (300 x 7.5 mm) (exclusion limits from 500 Da to 300 000 Da) at a flow rate of 0.5 mL/min. Column temperature was held at 50°C. The chromatograms were recorded with Chromeleon 7.2 software and Astra 7.1.0 software and analyzed using Originlab 2016 software. The dn/dc were determined experimentally. b) Hexafluoro-2-propanol (HFIP+ 0,05% KTFA) as the eluent (LCPO, Bordeaux, France). Measurements in HFIP were performed on an Ultimate 3000 system from Thermoscientific equipped with diode

array detector DAD. The system also included a multi-angle light scattering detector MALS and differential refractive index detector dRI from Wyatt technology. Polymers were separated on PL HFIP gel column (300 x 7.5 mm) (exclusion limit from 100 Da to 150 000 Da) at a flow rate of 0.8 mL/min. Column temperature was held at 40°C. The chromatograms were recorded with Chromeleon 7.2 software and Astra 7.1.0 software and analyzed using Originlab 2016 software. The molar mass was calculated using either a calibration curve or the dn/dc value. The calibration curve was performed with polystyrene standards with molar masses in the range 0.9-364 kg/mol. The dn/dc were determined experimentally. MALDI-MS spectra were performed at CESAMO facility (Bordeaux, France) on an Autoflex maX TOF mass spectrometer equipped with a frequency tripled Nd:YAG laser emitting at 355 nm. Spectra were recorded in the positive-ion mode using the reflectron and with an accelerating voltage of 19 kV. For MALDI-MS analyses, polypeptoid deposits were prepared according to the two following recipes: 1) Polypeptoid and the cationic agent (NaI) were dissolved in methanol at 10 mg/mL. The α-CHCA matrix (α-cyano-4-hydroxycinnamic acid) solution was prepared by dissolving 10 mg in 1 mL of methanol and the solutions were combined in a 10:1:1 volume ratio (matrix:sample:salt). One microliter of this solution was deposited onto the grid and vacuum-dried before analysis. 2) the polymer and the cationic agent (NaI) were dissolved in methanol at 10 mg/mL. The dithranol matrix solution was prepared by dissolving 10 mg in 1 mL of dichloromethane and the solutions were combined in a 10:1:1 volume ratio (matrix:sample:salt). One microliter of this solution was deposited onto the grid and vacuum-dried before analysis.

Kinetics study. The analyzed compounds were dissolved in anhydrous solvent in the Schlenk vessel under an argon atmosphere at 20 °C at 0.4 M. Samples (50 µL) were taken with an argon purged syringe at predetermined intervals. The samples were dropped onto the ATR unit of the IR spectrometer and measured in the range of 400-4000 cm-1. The monomer conversion was followed using the band height of the C=O stretching at about 1850 cm-1, whose increase is linear in the examined concentration range. The initial value for the band at 100% conversion was corrected using THF or DMF as blank. Samples were taken until no further change in band height was observed in order to determine the monomer conversion and corresponding reaction times (the point were no further change occurred was assumed to correspond to 100% conversion). Data analysis was carried out using Origin 2016 software (OriginLab).

Molecular modelling. Calculation were performed usually on 4 cores on a “home-made” setup equipped with 4 Dual Intel Xeon Silver 4214 2.2GHz, 3.2GHz Turbo, 12C and 8x32GB DDR4 2933MHz RDIMM ECC Memory. Computations were performed on NNCA monomers, allylamine and methyl ester N-alkylated glycines structures. The structures were built and pre-optimized with the software Chemcraft. Geometry optimizations of the resulting structures were performed with the B3LYP density functional37 and the 6-311+G(d,p) basis set with the Orca program.38 The bulk solvent effects were described with the integral equation formalism polarizable continuum Model (IEFPCM) with DMF as solvent.39 Vibrational frequencies were computed to confirm the convergence to local minima and to calculate the unscaled zero-point-energy (ZPE), the Gibb’s free energy at 298 K as well as the HOMO and the LUMO energies.

Synthetic Procedures.

The N-alkylated-N-Boc-glycine NNCA precursors were prepared according to the procedures presented in the ESI. In a second step, the synthesis of the different NCAs were operated using the Leuchs method, as previously reported18 and detailed below.

Synthesis of sarcosine-NCA (N-Me-NCA 1): In a clean, dry Schlenk flask N-methyl-N-Boc-glycine (2.27 g, 12 mmol, 1 equiv.) was dried for 10 min in vacuo, then 14 mL anhydrous THF was added, followed by thionyl chloride (0.88 mL, 12 mmol, 1 equiv.). The reaction was stirred for 2 h at RT. The solvent was evaporated in vacuo, and the residue was washed with 3×30 mL anhydrous hexane, filtered using a cannula, and the powder was dried. Then 7.5 mL of anhydrous DCM were added and the suspension was filtered over previously dried Celite. The system was washed with 5×15 mL of anhydrous DCM and the solvent was evaporated in vacuo. The product was obtained as a white solid yielding 72% (0.99 g). C4H5NO3, M = 115.09 g/mol, MS ESI+: m/z = 116.03 [M+H]+. 1H-NMR, CDCl3 400 MHz δ: 3.03 (s, 3H, CH3), 4.12 (s, 2H, CH2). 13C-NMR, CDCl3 100 MHz δ: 30.41 (s, CH3), 51.04 (s, CH2), 152.45 (s, NCOO), 165.45 (s, COO). Both proton and carbon NMR were in agreement with previously reported syntheses.7,12

Synthesis of N-isopropyl-NCA (N-iPr-NCA 2): The product was prepared according to the synthesis of N-Me-NCA 1 and isolated as a white solid in 89% yield (0.99 g) starting from N-isopropyl-N-Boc-glycine (1.7 g, 7.8 mmol). C6H9NO3, MW = 143.14 g/mol, MS (CI+): m/z= 144.06 [M+H]+, 1H-NMR, CDCl3 400 MHz δ: 1.25 (d, 6H, J=6.8 Hz, CH3), 4.04 (s, 2H, CH2), 4.27 (hept, 1H, J=6.8 Hz, CH). 13C-NMR, CDCl3 100 MHz δ: 20.05 (2CH3), 44.89 (CH), 45.31 (CH2), 151.35 (NCOO), 165.93 (COO). Both 1H- and 13C-NMR were in agreement with previously reported syntheses.30

Synthesis of N-cyclohexyl-NCA (N-ch-NCA 3): The product was prepared according to the synthesis of N-Me-NCA 1 and isolated as a white power in 80% yield (0.57 g) starting from N-cyclohexyl-N-Boc-glycine (1 g, 3.9 mmol). C9H13NO3, MW = 183.21 g/mol, MS (CI+): m/z= 184.09 [M+H]+¬, 1H-NMR, CDCl3 400 MHz δ: 1.02-1.19 (m, 1H, CH), 1.25-1.49 (m, 4H, 2CH2), 1.65-1.76 (m, 1H, CH), 1.98-1.77 (m, 4H, 2CH2), 3.72-3.97 (m,1H, NCH), 4.04 (s, 2H, CH2). 13C-NMR, CDCl3 100 MHz δ: 25.09 (CH2), 25.18 (2CH2), 30.47 (2CH2), 45.63 (CH), 52.71 (CH2), 151.44 (NCOO), 166.13 (COO).

Synthesis of N-benzyl-NCA (N-Bz-NCA 4): The product was prepared according to the synthesis of N-Me-NCA 1 and isolated as a yellowish crystalline powder in 79% yield (0.57 g) starting from N-benzyl-N-Boc-glycine (1 g, 3.8 mmol). C10H9NO3, MW = 191.19 g/mol, MS (CI+): m/z= 192.06 [M+H]+¬, 1H-NMR, CDCl3 400 MHz δ: 3.95 (s, 2H, CH2), 4.57 (s, 2H, CH2), 7.27-7.44 (m, 5H, Ar). 13C-NMR, CDCl3 100 MHz δ: 47.67 (CH2), 48.40 (CH2), 128.39 (2CH), 128.70 (CH), 129.34 (2CH), 133.85 (C), 152.29 (NCOO), 165.39 (COO). Both proton and carbon NMR were in agreement with a previously reported synthesis.40

Synthesis of N-gem-trifluoroethyl-NCA (N-gtfEt-NCA 5): The product was prepared according to the synthesis of N-Me-NCA 1 and isolated as a white powder in 46% yield (0.32 g) starting from N-(trifluoroethyl)-N-Boc-glycine (1 g, 3.9 mmol). C5H4 F3NO3, MW = 183.01 g/mol, MS (CI+): m/z= 184.02 [M+H]+, 1H-NMR, CDCl3 400 MHz δ: 4.03 (q, 2H, J=8.5 Hz, CH2), 4.92 (s, 2H, CH2). 13C-NMR, CDCl3 100 MHz δ: 45.17 (q, J=35.9 Hz, CH2), 49.87 (CH2), 123.43 (q, J=279 Hz, CH2), 152.16 (NCOO), 163.74 (COO).

Synthesis of N-(2-methylthio)ethyl-NCA (N-MeSEt-NCA 6): The product was prepared according to the synthesis of N-Me-NCA 1 and isolated as a yellow viscous oil in 71% yield (0.49 g) starting from N-[2-(methylthio)ethyl]-N-Boc-glycine (1 g, 4.0 mmol). C6H9NO3S, MW = 175.20 g/mol, MS (CI+): m/z= 176.03 [M+H]+¬¬, 1H-NMR, CDCl3 400 MHz δ: 2.16 (s, 3H, CH3), 2.76 (t, 2H, J=6.4 Hz, CH2), 3.63 (t, 2H, J=6.4 Hz, CH2), 4.24 (s, 2H, CH2). 13C-NMR,

CDCl3 100 MHz δ: 15.29 (CH3), 32.00 (CH2), 41.99 (CH2), 49.70 (CH2), 152.36 (NCOO), 165.34 (COO).
Synthesis of N-p-nitrobenzyl-NCA (N-pnBz-NCA 7): The product was prepared according to the synthesis of N-Me-NCA 1 and was isolated as a yellowish powder in 65% yield (0.49 g) starting from N-(p-nitrobenzyl)-N-Boc-glycine (1 g, 3.2 mmol). C10H8N2O5, MW = 236.18 g/mol, MS (CI+): m/z= 237.05 [M+H]+, 1H-NMR, CDCl3 400 MHz δ: 4.03 (s, 2H, CH2), 4.68 (s, 2H, CH2), 7.43-7.55 (m, 2H, CH Ar), 8.20-8.37 (m, 2H, CH Ar). 13C-NMR, CDCl3 100 MHz δ: 47.71 (CH2), 48.43 (CH2), 128.42 (2CH), 128.89 (C), 129.36 (2CH), 133.85 (C), 152.29 (NCOO), 165.37 (COO).
Synthesis of N-benzyloxycarbonyl-aminoethyl-NCA (N-ZNEt-NCA 8): The product was prepared according to the synthesis of N-Me-NCA 1 and isolated as a white powder in 47% yield (0.63 g) starting from N-(Cbz-aminoethyl)-N-Boc-glycine (1.7 g, 4.8 mmol). C13H14N2O5, MW = 278.26 g/mol, MS (CI+): m/z= 279.09 [M+H]+, 1H-NMR, CDCl3 400 MHz δ: 3.36-3.48 (m, 4H, 2CH2), 4.16 (s, 2H, CH2), 5.07 (s, 2H, CH2), 5.29 (s, 1H, NH), 7.31-7.37 (m, 5H, Ar). 13C-NMR, CDCl3 100 MHz δ: 38.41 (CH2), 43.90 (CH2), 49.39 (CH2), 67.30 (CH2), 128.21 (2CH), 128.46 (CH), 128.75 (2CH), 136.24 (C), 153.00 (NCOO), 157.09 (NCOO), 165.42 (COO).
Synthesis of N-benzyloxycarbonyl-aminobutyl-NCA (N-ZNBu-NCA 9): The product was prepared according to the synthesis of N-Me-NCA 1 and isolated as a white powder in 44% yield (0.6 g) starting from N-(Cbz-aminobutyl)-N-Boc-glycine (1.7 g, 4.5 mmol). C15H18N2O5, MW = 306.32 g/mol, MS (CI+): m/z= 307.12 [M+H]+, 1H-NMR, CDCl3 400 MHz δ: 1.38-1.75 (br, 4H, 2CH2), 3.25 (q, 2H, J=6.4 Hz, CH2), 3.42 (t, 2H, J=7.1 Hz, CH2), 4.04 (s, 2H, CH2), 4.84 (s, 1H, NH), 5.09 (s, 2H, CH2), 7.31-7.39 (m, 5H, Ar). 13C-NMR, CDCl3 100 MHz δ: 24.39 (CH2), 27.16 (CH2), 40.24 (CH2), 43.31 (CH2), 49.05 (CH2), 66.76 (CH2), 128.11 (2CH), 128.25 (CH), 128.62 (2CH), 136.63 (C), 152.28 (NCOO), 156.69 (NCOO), 165.68 (s, COO).
Synthesis of N-propyl-NCA (N-Pr-NCA 10): The product was prepared according to the synthesis of N-Me-NCA 1 and isolated as a colorless liquid in 60% yield (0.79 g). 1H NMR, CDCl3 400 MHz δ: 0.97 (t, 3H, CH3), 1.63 (m, 2H, CH2), 3.37 (t, 2H, CH2), 4.10 (s, 2H, CH2). Proton NMR were in agreement with a previously reported synthesis.7
Glycine and N-methylalanine NCA syntheses are developed in the ESI.
Preparation of polymers. The polymers were prepared in DMF and at a monomer concentration of 0.4 M. To compare the kinetics, a degree of polymerization (M/I) of 20 was implemented using allylamine. The details specific to each polymerization are given below.
Synthesis of poly(N-methyl)glycine (PSar 1'). Sarcosine-NCA monomer (0.2 g, 1.74x10-3 mol) was weighed in a glovebox under pure argon, introduced in a flame-dried Schlenk vessel, and dissolved in 4.3 mL of anhydrous DMF. A solution of allylamine (0.5 M in DMF) was prepared and 174 µL (8.69x10-5 mol) were added with an argon purged syringe. The solution was stirred at RT under argon until completion. The polymer was then recovered as a white solid by precipitation in diethyl ether and dried in vacuo. Yield: 60% (0.074 g). 1H-NMR DMSO-d6 400 MHz δ (ppm): 2.50-3.10 (m, 69H, CH3), 3.62-3.80 (m, 2H, CH2 allylamine), 3.80-4.42 (m, 42H, CH2), 5.0-5.20 (m, 2H, CH2 allylamine), 5.64-5.82 (m, 1H, CH allylamine). NH terminal missing in these experimental conditions.
Synthesis of poly(N-isopropyl)glycine (PNiPr 2'). The synthesis was similar to the one described for 1'. The polymer was recovered as a white solid in 60% yield (72 mg) starting from N-iPr-NCA (174 mg, 1.22x10-3 mol). 1H-NMR TFA-d 400 MHz δ (ppm): 1.03-1.57 (m, 150H, CH3), 3.16-4.14 (m, 24H, CH), 4.52-4.15 (m, 55H, CH2), 4.78-4.91 (m, 2H, CH2

allylamine), 5.25 (m, 1H, CH allylamine). NH terminal missing in these experimental conditions.

Synthesis of poly(N-cyclohexyl)glycine (PNch 3'). The synthesis was similar to the one described for 1'. The polymer was recovered as a white solid in 79% yield (0.12 g) starting from N-chG-NCA (0.2 g, 1.09x10-3 mol). 1H-NMR TFA-d 400 MHz δ (ppm): 1.08-2.32 (m, 275H, CH2), 3.27-3.69 (m, 26H, CH), 3.76-3.86 (m, 2H, CH2 allylamine), 4.08-4.59 (m, 57H, CH2), 5.14-5.40 (m, 2H, CH2 allylamine), 5.90-5.71 (m, 1H, CH allylamine). NH terminal missing in these experimental conditions.

Synthesis of poly(N-benzyl)glycine (PNBz 4'). The synthesis was similar to the one described for 1'. The polymer was recovered as a yellowish solid in 68% yield (66 mg) starting from N-Bz-NCA (0.126 g, 6.59x10-4 mmol). 1H-NMR TFA-d 400 MHz δ (ppm): 3.71-3.91 (m, 2H, CH2 allylamine), 3.96-4.81 (m, 70H, CH2+CH2Ar), 5.00-5.20 (m, 2H, CH2 allylamine), 5.57-5.76 (m, 1H, CH allylamine), 6.73-7.51 (m, 85H, Ar). NH terminal missing in these experimental conditions.

Synthesis of poly(N-(gem-trifluoroethyl))glycine (PNtfg 5'). We did not succeed in polymerizing this monomer (see discussion section).

Synthesis of poly(N-(2-methylthioethyl))glycine (PNmt 6'). The synthesis was similar to the one described for 1'. The polymer was recovered as a white solid in 41% yield (0.12 g) starting from N-mtG-NCA (400 mg, 2.28x10-3 mol). 1H-NMR DMSO-d6 400 MHz δ (ppm): 1.98-2.23 (m, 86H, CH3), 2.53-2.72 (m, 47H, CH3), 3.67-3.79 (m, 2H, CH2 allylamine), 3.86-4.70 (m, 49H, CH2), 4.97-5.29 (m, 2H, CH2 allylamine), 5.66-5.93 (m, 1H, CH allylamine). NH terminal missing in these experimental conditions.

Synthesis of poly(N-(p-nitrobenzyl))glycine (PNpBz 7'). The synthesis was similar to the one described for 1'. The polymer was recovered as a yellowish solid in 69% yield (88 mg) starting from N-pbz-NCA (157 mg, 6.62x10-4 mol). 1H-NMR DMSO-d6 400 MHz δ (ppm): 3.53-3.71 (m, 2H, CH2 allylamine), 3.78-4.84 (m, 72H, CH2+CH2Ar), 4.91-5.15 (m, 2H, CH2 allylamine), 5.56-5.78 (m, 1H, CH allylamine), 7.23-8.37 (m, 92H, Ar). NH terminal missing in these experimental conditions.

Synthesis of poly(N-(Cbz-2-aminoethyl))glycine (PNzae 8'). The synthesis was similar to the one described for 1'. The polymer was recovered as a yellowish solid in 52% yield (81 mg) starting from N-Zae-NCA (185 mg, 6.65x10-4 mol). 1H-NMR DMSO-d6 400 MHz δ (ppm): 2.92-3.60 (m, 86H, 2CH2), 3.78-4.50 (m, 34H, CH2) 4.84-5.21 (m, 40, CH2), 5.59-5.90 (m, 1H, CH2 allylamine), 7.04-7.49 (m, 104H, Ar+NHCO). NH terminal missing in these experimental conditions.

Synthesis of poly(N-(Cbz-2-aminobutyl))glycine (PNzab 9'). The synthesis was similar to the one described for 1'. The polymer was recovered as a yellowish solid in 64% yield (110 mg) starting from N-Zab-NCA (0.203 g, 6.63x10-4 mol). 1H-NMR DMSO-d6 400 MHz δ (ppm): 1.20-1.63 (m, 110H, 2CH2), 2.91-3.06 (m, 41H, CH2), 3.10-3.36 (m, 40H, CH2), 3.60-4.47 (m, 39H, CH2), 4.92-5.12 (m, 51H, CH2), 5.65-5.90 (m, 1H, CH allylamine), 7.05-7.46 (m, 156H, Ar+NHCO). NH terminal missing in these experimental conditions.

Synthesis of poly(N-propyl)glycine (PNPr 10'). The synthesis was similar to the one described for 1'. The polymer was recovered as a white solid in 58% yield (0.08 g) starting from N-Pr-NCA (200 mg, 1.40x10-3 mol). 1H-NMR D2Oi 400MHz δ (ppm): 0.77-1.05 (m, 38H, CH2), 1.37-1.94 (m, 59H, CH3), 3.10-3.54 (m, 40H, CH2), 3.80-3.91 (m, 2H, CH2 allylamine), 3.97-4.62 (m, 39H, CH2), 5.16 (s, 2H, CH2 allylamine), 5.86 (s, 1H, CH allylamine), 7.49 (s, 1H, NH terminal).

Synthesis of polyglycine, poly(L-alanine) and poly(N-methyl-L-alanine). The detailed procedures of these 3 syntheses are developed in the ESI.

Results and discussion

Even though they were first prepared over 100 years ago, the synthesis of NCAs from amino acids remains an important topic of research.41 The first examples of NCA synthesis were described with thionyl chloride ($SOCl_2$)10 and various NCA compounds were successfully prepared from N-carboxybenzyl- or N-carboxyethyl-substituted amino acids including for instance glycine, L-phenylalanine and L-leucine.10,42,43 Reacting N-substituted amino acids with other chlorinated or brominated electrophilic reagents, such as PCl_5 and PBr_3 also afforded NCA including L-ornithine, L-phenylalanine, L-alanine, L-lysine and L-valine derivatives.44,45 This approach, called the Leuchs method, is an alternative to the Fuchs' and Farthing's method consisting in the reaction of unsubstituted amino acids with an excess of phosgene or its derivatives (diphosgene and triphosgene).46–48 The knowledge developed from the synthesis of N-carboxyanhydrides can be extended to the synthesis of N-alkylated-NCA. Several routes were investigated with NNCAs. For example, N-(benzyloxycarbonyl)glycine derivatives bearing various N-alkyl substituents were reacted with acetyl chloride and acetic anhydride.7,22,30 Other reagents such as $PBr_3$11,49 or PCl_3 were used to prepared NNCAs from N-(tert-butoxycarbonyl)glycine derivatives bearing a wide range of alkyl and oligo(ethylene glycol) groups.6,9,12,13,29,34,50–52 Although both approaches can be considered to be variations of the Leuchs method, the original protocol using $SOCl_2$ has scarcely been developed: early studies demonstrated the preparation of N-phenylglycine-NCA43 and more recent ones presented the preparation of lysine-, phenylalanine-like- and sarcosine-NCAs.18 Therefore, we decided in this work to focus on the use of the Leuchs method with $SOCl_2$ (scheme 1).

We first designed a method to prepare a library of N-alkylated-N-Boc-glycines, as NNCA precursors (scheme 1a).10 The N-alkyl-N-Boc intermediates are formed in a one-pot procedure consisting of i) the formation of N-alkyl-glycine (not isolated) from reductive amination of glyoxylic acid with various primary alkylamines and ii) Boc protection (using Boc_2O) of the secondary amine without prior purification. We initially prepared five N-alkylated-N-Boc-glycine derivatives bearing alkyl side chains of increasing lipophilicity (methyl, propyl, isopropyl, cyclohexyl and benzyl groups). All compounds were isolated with good purity (60-77% yields) after liquid-liquid extractions, avoiding the use of chromatographic purification (see Supporting information). We then prepared five more N-alkyl-N-Boc-glycine derivatives bearing functionalized side chains (gem-trifluoroethyl, methylthioethyl and p-nitro-benzyl and two N-protected aminoalkyl groups). The last two intermediates were prepared from commercial 1,2-diaminoethyl- or 1,4-diaminobutyl-containing reagents on which one amine function was protected by a benzyloxycarbamate (Cbz or Z) group (see Supporting information). Finally, N-Boc derivatives of N-methyl-L-alanine and glycine were purchased or prepared according to a different method (see experimental part and esi). These synthetic efforts resulted in a library of 12 N-Boc-aminoacid derivatives, enabling further study of i) the formation of each NNCA via the Leuchs reaction and ii) their ROP kinetics, initiated with allylamine (scheme 2).

Scheme 2. The different NNCA monomers that were prepared and studied (polymerization kinetics) considering several key effects brought by the N-alkylation.

Kinetics of NNCA formation via the Leuchs method.

Despite growing interest in the synthesis of NCA monomers, the effect of the N-alkyl side chains on the kinetics of the N-carboxyanhydride ring formation has not really been described. We addressed this question by i) conducting a kinetic study of the preparation of 10 N-alkylated NCA monomers bearing various alkyl side chains via the Leuchs method using SOCl2 and ii) achieving DFT calculation to optimize the geometry of the monomers and to determine the LUMO energy level of each different NNCA ring (see table 1 and exp. section). The rates of NNCA formation were measured by monitoring the IR absorbances corresponding to the C=O bond in NCO (1850 cm-1 stretching) (figure 1). Effects of steric hindrance (NNCAs 1-4), electron withdrawing or donating effects (NNCA 5-9) and carbon chain length (NNCA 8, 9) were therefore evaluated.

Figure 1. Monitoring of the NNCA formation. A) Synthesis scheme and B) evolution of the C=O (NCO) stretching signal during the NNCA formation monitored by FITR at 1850 cm-1. NNCA formation were carried out at RT (20 °C) in THF solution (0.4 M). We first studied the effect of the different aliphatic side chains with increasing steric hindrance by following the preparation of N-methyl-glycine-NCA (Sar-NCA 1), N-isopropyl-glycine-NCA (N-iPr-NCA 2), N-cyclohexyl-glycine-NCA (N-ch-NCA 3) and N-benzyl-glycine NCA (N-Bz-NCA 4, figure S1) from the corresponding N-Boc glycine derivatives. We used the conversion at 50-60 min to determine the speed of the NNCA formation. We observed a decrease in NNCA conversion when a bulkier alkyl substituent was introduced. First, Sar-NCA showed the highest conversion of 99.4% at 58 min, while N-ch-NCA presented a lower conversion of 73.2% at 50 min, followed by N-Bz-NCA (82.6% conversion at 60 min, table 1). The slight difference observed between N-cyclohexyl and Sar-NCA was attributed to a conformational effect of the cyclohexyl inducing a higher steric hindrance while the higher conversion rate of N-benzyl could be explained by an inductive effect. Second, formation of N-iPr-NCA was associated with the lowest conversion rate of 56.7% at 60 min, a behavior that was attributed to stronger steric hindrance in the vicinity of the amine function that ring-closed, as confirmed by the calculated value of the LUMO energy (-0.93 eV) found in the same range as the LUMO energy values found for 1, 3 and 4.35

Table 1. Summary of the kinetics obtained for the synthesis of NNCA using the Leuchs method.

NNCA	Yield (%) Conversion (%) at 60 min	LUMO (eV)	NNCA	Yield (%) Conversion (%) at 60 min	LUMO (eV)
Sarcosine-NCA (1)	72	99.4a	- 0.99		
N-MeSEt–NCA (6)	71	74.5	- 1.00		

N-iPr-NCA (2)	89	56.7	- 0.93
N-pnBz–NCA (7)	65	98.5	- 0.98
N-ch-NCA (3)	80	73.2b	- 0.93
N-ZNEt–NCA (8)	47	68.7	- 1.02
N-Bz-NCA (4)	79	82.6	- 1.02
N-ZNBu–NCA (9)	44	41.9c	- 0.98
N-gtfEt–NCA (5)	46	46.6	- 1.14

[a] recorded at 58 min, [b] recorded at 50 min, [c] recorded at 54.6 min

We then studied the effect of different heteroatom-containing side chains by following the preparation of N-gem-trifluoroethyl-glycine NCA (N-gtfEt-NCA 5), N-methylthioethyl-glycine NCA (N-MeSEt-NCA 6), N-para-nitrobenzyl-glycine NCA (N-pnBz-NCA 7), N-Z-aminoethyl-glycine NCA (N-ZNEt-NCA 8) and N-Z-aminobutyl-glycine NCA (N-ZNBu-NCA 9). The N-Boc-glycine intermediates when they were mixed with SOCl2 were all fully converted to their corresponding NNCAs (see figure S1). The conversion rates of the cyclization reaction were not significantly influenced by the presence of the heteroatoms, as evidenced first by the N-pnBz-NCA formation (98.5% conversion at 60 min, table 1) that was found in the same range as the one found for N-Bz-NCA formation (82.6% conversion at 60 min). As an exception to this observation, the N-trifluoroethyl derivative showed that the formation of the corresponding NNCA monomer was significantly slowed down (46.6% conversion at 60 min), an effect that we attributed to the very strong attractive effect of the fluorine atoms toward the electron density on the nitrogen atom during ring-closing. This electronic effect was further confirmed by the calculated LUMO energy value that was found at -1.14 eV, the lowest value calculated for any of the NNCA monomers (see table 1).

In summary, we prepared a small library of NNCAs using an optimized protocol involving a 2-step synthesis: 1) reductive amination between primary amines and glyoxylic acid followed by the N-Boc protection in a “one-pot” procedure (yields 60-77%)) without any chromatography purification; 2) cyclization using the Leuchs method with SOCl2 (yields 44-89%). A kinetic study of the cyclization step established that bulkier alkyl groups slowed down the NNCA formation (time range: 1 to 7 h to complete conversion), while electronic effects were negligible.

Kinetics of the ring opening polymerization:

During the NCA synthesis, the Leuchs method releases HCl and chlorinated byproducts that may persist even after recrystallization.53–56 Acidic contaminants retard the ROP process,7 and their elimination is therefore important to obtain an efficient and reproducible polymerization.15 We found that filtration over celite could successfully remove chlorides reflected in 0.6-3.8 fold increase in the first-order rate coefficients and afforded the fastest polymerization of Sar-NCA (k = 0.53 h-1, see figure S2) in comparison to the non-purified and crystallized monomers at DP = 100. Therefore, we employed this method to purify all our NNCA monomers in the different ROP kinetic studies.

N-alkylation versus C-alkylation. The N-alkylation of NNCAs or the C-alkylation of NCAs strongly influences the polymerization kinetics.57,58 In this work, we investigated the ROP kinetics of glycine NCA, Sar-NCA and N-methyl-alanine-NNCAs in DMF at 0.4 M under argon

and at a targeted ratio of Monomer/Initiator (M/I) = 20, using allylamine as initiator. The kinetics were determined by FTIR monitoring : decrease of the signal of the carbonyl stretching band (C=O) at 1850 cm-1. After almost 2 days, all polymerization reactions had reached complete conversion and the polymers were characterized by 1H-NMR and MALDI-TOF (see ESI figures S3-5, S15-17). To determine the first-order rate coefficients, the data were fitted by NNLS using an exponential decay (Mt = 1 – exp(-k.(t-t0)), where the Mt is the intensity at t time. The resulting plots were consistent with first order kinetics for all 3 ROP reactions (figure 2 and table 2).

Figure 2. Kinetics of the ROP process for NNCA in DMF 0.4 M having an M/I = 20 varying the methylated position on different peptidomimetic polymers. *PNMeAla is depicted in hours.

Table 2. Kinetic rates of the ROP of NNCA with different methylations

Polymer	kobs (h-1)	t0 (h)	Mn (kg/mol)	Đ
PGly	9.54 ± 0.42	-0.058 ±0.003	n.aa	-
PSar	4.02 ± 0.36	-0.008 ± 0.013	1.0 b	1.03
PNMeAla	0.084 ± 0.006	1.2±0.2	2.7c	1.18

[a] The polymer was not soluble even in HFIP. [b] The Mn was determined by SEC in DMF. [c] The Mn was determined by SEC in HFIP.

Steric versus electronic effect of the N-alkylation. The methylation data showed that the effect of N-alkylation was more complex than just steric hindrance. Previous works have suggested that the electronic effect could be as strong as the steric hindrance in some cases. For instance, the recent ROP of N-(3-tert-butoxy-3-oxopropyl)-NCA demonstrated the relatively fast preparation of polypeptoid analogues of polyglutamic acid with a propagation rate constant, kp, of 181 M-1·h-1.5 This work provided a unique opportunity to rationalize the influence of different N-substitutions on the ROP kinetics of NNCAs. We set up the polymerization of NNCA using allylamine as initiator (targeting a M/I = 20) in anhydrous DMF under argon. The reaction mixtures were monitored by FTIR: the direct observation of the disappearance of the NNCA stretching band indicated that the reaction time to reach full conversion varied significantly, ranging from 3 h for PSar (1') to more than 12 days for PNch (3'). Upon ROP, all the homopolymers were purified by precipitation using diethyl ether. The yields were good for almost all polypeptoids (60 to 80% of the mass was recovered) except for PNmt (6') that afforded a low yield of 41%. This low yield was attributed to several precipitation steps performed on an oily product for which a high affinity to DMF was observed. All the polymers were fully characterized by 1H-NMR analyses, size exclusion chromatography (SEC), MALDI TOF and FTIR analyses (see figures S4-25, except MALDI TOF for PNiPr (2') because we could not determine it using either dithranol or α-CHCA). Solubility was a very important parameter for the characterization and most of the copolymers were soluble in polar solvents or in water (table S1). Molar masses determined by SEC showed a lower value than the theoretical one in most cases which was also found in previous works.7,29,59 Despite this deviation, the polypeptoids synthesized presented low dispersities in the range ĐM = 1.08-1.30, except for PNpBz (7') (ĐM=1.91). MALDI TOF

analysis confirmed that allylamine efficiently initiated the ROP and FTIR analyses revealed the expected amide I stretching band at ~1650 cm-1 (figure S25).7

With aliphatic groups, the experimental kinetics of NNCA monitored by FTIR were highly influenced by steric hindrance. For instance, PNPr (10') reached full conversion after 48 h with a kinetic constant of k = 6.3 ± 0.4 x10-2 h-1 (table 3) that was 64 times lower than the kinetic constant of the ROP forming PSar (1', k = 4.0 ± 0.4 h-1). This influence was even more pronounced when we performed the ROP of more sterically hindered monomers such as N-iPr-NCA and N-ch-NCA. The full conversion into PNiPr (2') was reached after 15 days and into PNch (3') after 20 days respectively (figure 3). Comparatively, the steric effect was reduced during the synthesis of PNPr (10'). The effect of such steric hindrance was surprising when we monitored the polymerization of N-benzyl-NCA towards PNBz (4', k = 1.5 ± 0.08 x 10-2 h-1). These results showed that the steric hindrance was significantly counterbalanced by another effect attributable to the benzyl group.

We also followed the ROP of several NNCAs with heteroatoms on their side chains (figure 3). First, we studied the ROP kinetics of N-pnBz-NCA that possesses a nitro electron-withdrawing group, to compare it with the N-benzyl side chain (N-Bz-NCA, figure 3). Inttersestingly, after about 15 days of reaction, we reached a full conversion and monitored a zero-order kinetic rate of k = 26 ± 1.0 x10-2 mol·L-1·h-1 (table 3). We thus noticed that PNpBz (7') was slower (25.7% conversion at 50 h) as compared with PNBz (4', 54% conversion at 48 h), confirming that the electron withdrawing effect can strongly influence the ROP reactivity. This effect increased the electron density on the nitrogen atom and thus its nucleophilicity: it is this same effect that is at the origin of a higher pKa for secondary amines compared to primary amines.60

Table 3. Characterization of the polypeptides and polypeptoids bearing different N-alkyl groups.

Polymer	Reaction order		kobs (x10-2 h-1)		t0 (h)	Mn (kg/mol)	ĐM
PSar (1')	First	402 ± 36	-0.008 ± 0.01	1.0	1.03		
PNiPr (2')	Zero-likea	0.33 ± 0.02	-10 ± 2	1.5	1.16		
PNch (3')	Zero-likea	0.27 ± 0.02	-12 ± 3	0.8	1.29		
PNBz (4')	First	1.55 ± 0.03	-0.4 ± 0.3	1.6	1.19		
PNmt (6')	First	2.4 ± 0.2	-0.9 ± 0.7	5.4	1.22		
PNpBz (7')	Zero-likea	0.26 ± 0.01	10 ± 1	4.0	1.91		
PNzae (8')	Zero-likea	0.94 ± 0.04	-9 ± 3	1.0 ± 0.3		1.29	
PNzab (9')	First	3.3 ± 0.1	-3.0 ± 0.3	5.6	1.08		
PNPr (10')	First	6.3 ± 0.4	0.4 ± 0.4	2.0	1.09		

[a] units of mol·L-1·h-1

It was also interesting to compare the ROP kinetics of N-benzylated-NCAs with NNCA monomers bearing ethyl substituents with a N-methylthioether (N-MeSEt-NCA) or a N-gem-trifluoroethyl functional group (N-gtfEt-NCA). The formation of PNmt 6' (k = 2.4 ± 0.2 x10-2 h-1) was 1.5 times faster than PNBz 4' (figure 3), an effect we attributed to enhanced nucleophilicity of the secondary amine during the propagation. In marked contrast, the PNgtfEt (5') took more than two weeks to achieve less than 5% of conversion (data not shown). This result indicated that electron-withdrawing groups (negative inductive effect) could strongly decrease the nucleophilicity during the propagation and consequently the ROP kinetics.

Finally, we also compared the ROP kinetics of N-ZNEt- and N-ZNBu-NCAs. Interestingly, PNZae (8') presented a zero-order kinetics profile (figure 3) whereas PNzab (9') showed the characteristic of a first-order kinetics profile reaching full conversion in both cases at 135 h. NNCA was consumed at the same speed as the CO2 was released, presenting an apparent zero-order for PNzae 8' (k = 0.94 ± 0.04 mol·L-1·h-1) while in the case of PNzab 9', the longer alkyl chain enhanced the constant rate (k = 3.3 ± 0.1 x10-2 h-1) when we compared with PNBz 4' (improvement of 2.1 fold). Notably, the kinetics were not fitted with the first-order model for 7' and 8', similarly to the kinetics from 2' (k = 0.33 ± 0.02· mol·L-1·h-1) and 3' (k = 0.27 ± 0.02 mol·L-1·h-1 figure 3B). Indeed we observed an induction period within the first hours of polymerization where the kinetics seemed to present a slower kinetics rate. We attributed this to two possible effects: 1) in the case of polymers 2' and 3' could be explained due to solubility effect because they become turbid during the course of the polymerization; 2) in the case of polymer 8' and 7' the effect could be due to the stabilization of the carbamate formed during the ROP (scheme S1), with the decarboxylation step the rate determining step.

To better estimate the influence of the electronic effect of the N-alkylation over the ROP of the proposed NNCA, DFT calculations of the HOMO energy levels of allylamine and of the propagating end extremities have also been performed using the B3LYP level of theory with the 6-311+G** basis set in DMF (CPCM, see experimental section). Based on the energy differences between these HOMO energy values and the LUMO of the different NNCA monomers, a higher reactivity is expected if the energy difference is smaller between these energy values (see figures 4 and S26). The difference observed with the experimental kinetics could then be attributed to another effect such as the steric hindrance. These DFT calculations showed for instance that the electronic effect had a significant influence for the propagation steps (figure 4) but not for the initiation steps (figure S26).

First, theoretical calculations indicated that for the propagation steps, N-alkylation activated the reactivity significantly (5.98 eV for glycine as compared to 5.48 eV for sarcosine). As this electronic effect was not increased with C-methylation, the DFT calculation confirmed that the slower kinetics during PNMeAla formation were attributable to the steric hindrance. Then, with aliphatic groups (propyl, isopropyl, cyclohexyl and benzyl), LUMO-HOMO energy difference values were all in the same range (5.32 to 5.47 eV), and even indicative of a less reactive system with N-benzylation (5.47 eV). This was in marked contrast to ROP kinetics of 4 suggesting an enhanced propagation rate attributable to Pi-Pi stacking. Finally, positive and negative electronic effect were confirmed by molecular modelling for the other NNCAs bearing heteroatoms on their side chains notably, i) a strong inductive acceptor effect for the ROP of 5 (5.76 eV) and ii) significantly stronger inductive donor effects for 8 and 9 (5.26 and 5.32 eV respectively) as compared to N-methylation (5.48 eV).

Conclusions

The nature of the N-alkyl group on polypeptoids is an effective chemical lever to tune physico-chemical properties such as solubility, amphiphilicity, conformation and even crystallization behavior.1 This structural feature also influences the kinetics of NNCA monomer formation and further polymerization which are still poorly understood. In this work, we successfully prepared a library of 12 NCAs (including 5 original ones) using a methodology based on the Leuchs method. Kinetics of NNCA formation showed that the nature of the alkyl group, considering both steric and electronic effects, had a modest influence on the cyclization reaction (time to complete conversion ranging from 1 to 7 h). In contrast to NNCA formation, steric and electronic effects were important features in the ROP reaction, with bulky groups slowing down the kinetics of ROP while electron-donating groups accelerated it. In some cases, surprisingly, N-alkylation was also found to retard the CO2 release, leading to deviation from first-order order kinetic profiles. This study opens interesting perspectives to develop new catalytic systems based on our findings to further improve the preparation of polypeptoid polymers.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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ABBREVIATIONS

NCA (N-carboxyanhydrides), NNCA (N-alkylated carboxyanhydride), ROP (ring-opening polymerization), Me (methyl), Et (ethyl), Pr (propyl), iPr (isopropyl), Bz (benzyl), pnBz (para-nitrobenzyl), MeSEt or Nmt (2-methylthioethyl), gtfEt or Ntfg (gem-trifluoroethyl), ZNEt or Nzae (Cbz-aminoethyl), ZNBu or Nzab (Cbz-aminobutyl).

References

(1) Chan, B. A.; Xuan, S.; Li, A.; Simpson, J. M.; Sternhagen, G. L.; Yu, T.; Darvish, O. A.; Jiang, N.; Zhang, D. Polypeptoid Polymers: Synthesis, Characterization, and Properties. Biopolymers 2017, 109 (1), 1–25.

(2) Zhou, P.; Shen, T.; Ling, J. Synthesis and Properties of Polypeptoid-Containing Block Copolymers: A Review. Journal of Polymer Science 2021, 59 (23), 2946–2958.
(3) Fetsch, C.; Gaitzsch, J.; Messager, L.; Battaglia, G.; Luxenhofer, R. Self-Assembly of Amphiphilic Block Copolypeptoids - Micelles, Worms and Polymersomes. Scientific Reports 2016, 6, 1–13.
(4) Sternhagen, G. L.; Gupta, S.; Zhang, Y.; John, V.; Schneider, G. J.; Zhang, D. Solution Self-Assemblies of Sequence-Defined Ionic Peptoid Block Copolymers. J Am Chem Soc 2018, 140 (11), 4100–4109.
(5) Zhang, D.; Barrett, B.; Sternhagen, G. Controlled Ring-Opening Polymerization of N-(3-Tert-Butoxy-3-Oxopropyl) Glycine Derived N-Carboxyanhydrides towards Well-Defined Peptoid-Based Polyacids. Polymer Chemistry 2021, 547–563.
(6) Guo, L.; Li, J.; Brown, Z.; Ghale, K.; Zhang, D. Synthesis and Characterization of Cyclic and Linear Helical Poly(α-Peptoid)s by N-Heterocyclic Carbene-Mediated Ring-Opening Polymerizations of N-Substituted N-Carboxyanhydrides. Peptide Science 2011, 96 (5), 596–603.
(7) Fetsch, C.; Grossmann, A.; Holz, L.; Nawroth, J. F.; Luxenhofer, R. Polypeptoids from N-Substituted Glycine N-Carboxyanhydrides: Hydrophilic, Hydrophobic, and Amphiphilic Polymers with Poisson Distribution. Macromolecules 2011, 44 (17), 6746–6758.
(8) Fetsch, C.; Luxenhofer, R. Thermal Properties of Aliphatic Polypeptoids. Polymers (Basel) 2013, 5 (1), 112–127.
(9) Lee, C.-U.; Li, A.; Ghale, K.; Zhang, D. Crystallization and Melting Behaviors of Cyclic and Linear Polypeptoids with Alkyl Side Chains. Macromolecules 2013, 46 (20), 8213–8223.
(10) Leuchs, H. Ueber Die Glycin-Carbonsäure. Berichte der deutschen chemischen Gesellschaft 1906, 39 (1), 857–861.
(11) Kricheldorf, H. R.; Von Lossow, C.; Schwarz, G. Primary Amine-Initiated Polymerizations of Alanine-NCA and Sarcosine-NCA. Macromolecular Chemistry and Physics 2004, 205 (7), 918–924.
(12) Guo, L.; Zhang, D. Cyclic Poly(α-Peptoid)s and Their Block Copolymers from N-Heterocyclic Carbene-Mediated Ring-Opening Polymerizations of N-Substituted N-Carboxylanhydrides. J Am Chem Soc 2009, 131 (50), 18072–18074.
(13) Lahasky, S. H.; Hu, X.; Zhang, D. Thermoresponsive Poly(α-Peptoid)s: Tuning the Cloud Point Temperatures by Composition and Architecture. ACS Macro Letters 2012, 1 (5), 580–584.
(14) Laconde, G.; Amblard, M.; Martinez, J. Synthesis of a-Amino Acid N-Carboxyanhydrides. Organic Letters 2021, 23 (16), 6412–6416.
(15) Semple, J. E.; Sullivan, B.; Sill, K. N. Large-Scale Synthesis of α-Amino Acid-N-Carboxyanhydrides. Synthetic Communications 2017, 47 (1), 53–61.
(16) Bossion, A.; Nicolas, J. Synthesis of Poly(Asparagine-Co-Phenylalanine) Copolymers, Analogy with Thermosensitive Poly(Acrylamide-Co-Styrene) Copolymers and Formation of PEGylated Nanoparticles. European Polymer Journal 2020, 140, 110033.
(17) Tian, Z. Y.; Zhang, Z.; Wang, S.; Lu, H. A Moisture-Tolerant Route to Unprotected α/β-Amino Acid N-Carboxyanhydrides and Facile Synthesis of Hyperbranched Polypeptides. Nature Communications 2021, 12 (1), 1–11.
(18) Salas-Ambrosio, P.; Tronnet, A.; Since, M.; Bourgeade-Delmas, S.; Stigliani, J.-L.; Vax, A.; Lecommandoux, S.; Dupuy, B.; Verhaeghe, P.; Bonduelle, C. Cyclic Poly(α-Peptoid)s by Lithium Bis(Trimethylsilyl)Amide (LiHMDS)-Mediated Ring-Expansion Polymerization: Simple Access to Bioactive Backbones. J Am Chem Soc 2021, 143 (10), 3697–3702.

(19) Chen, K.; Wu, Y.; Wu, X.; Zhou, M.; Zhou, R.; Wang, J.; Xiao, X.; Yuan, Y.; Liu, R. Facile Synthesis of Polypeptoids Bearing Bulky Sidechains via Urea Accelerated Ring-Opening Polymerization of α-Amino Acid N -Substituted N -Carboxyanhydrides. Polym. Chem. 2022, No. 13, 420–426.
(20) Gebru, H.; Tianfo, G.; Li, Z. Guanidine Masked Catechol Initiator Promoted Ring-Opening Polymerization of Sarcosine N-Carboxyanhydride. International Journal of Polymer Analysis and Characterization 2020, 25 (4), 262–274.
(21) Dove, A. P. Controlled Ring-Opening Polymerisation of Cyclic Esters: Polymer Blocks in Self-Assembled Nanostructures. Chemical Communications 2008, No. 48, 6446–6470.
(22) Fetsch, C.; Luxenhofer, R. Highly Defined Multiblock Copolypeptoids: Pushing the Limits of Living Nucleophilic Ring-Opening Polymerization. Macromolecular Rapid Communications 2012, 33 (19), 1708–1713.
(23) Fu, X.; Xing, C.; Sun, J. Tunable LCST/UCST-Type Polypeptoids and Their Structure–Property Relationship. Biomacromolecules 2020, 21 (12), 4980–4988.
(24) Zhang, Y.; Heidari, Z.; Su, Y.; Yu, T.; Xuan, S.; Omarova, M.; Aydin, Y.; Dash, S.; Zhang, D.; John, V. Amphiphilic Polypeptoids Rupture Vesicle Bilayers to Form Peptoid-Lipid Fragments Effective in Enhancing Hydrophobic Drug Delivery. Langmuir 2019, 36 (47), 15335–15343.
(25) Zhu, L.; Simpson, J. M.; Xu, X.; He, H.; Zhang, D.; Yin, L. Cationic Polypeptoids with Optimized Molecular Characteristics toward Efficient Nonviral Gene Delivery. ACS Applied Materials & Interfaces 2017, 9 (28), 23476–23486.
(26) Secker, C.; Völkel, A.; Tiersch, B.; Koetz, J.; Schlaad, H. Thermo-Induced Aggregation and Crystallization of Block Copolypeptoids in Water. Macromolecules 2016, 49 (3), 979–985.
(27) Xuan, S.; Lee, C. U.; Chen, C.; Doyle, A. B.; Zhang, Y.; Guo, L.; John, V. T.; Hayes, D.; Zhang, D. Thermoreversible and Injectable ABC Polypeptoid Hydrogels: Controlling the Hydrogel Properties through Molecular Design. Chemistry of Materials 2016, 28 (3), 727–737.
(28) Gangloff, N.; Höferth, M.; Stepanenko, V.; Sochor, B.; Schummer, B.; Nickel, J.; Walles, H.; Hanke, R.; Würthner, F.; Zuckermann, R. N.; Luxenhofer, R. Linking Two Worlds in Polymer Chemistry: The Influence of Block Uniformity and Dispersity in Amphiphilic Block Copolypeptoids on Their Self-Assembly. Biopolymers 2019, 110 (4), 1–11.
(29) Robinson, J. W.; Schlaad, H. A Versatile Polypeptoid Platform Based on N-Allyl Glycine. Chemical Communications 2012, 48 (63), 7835–7837.
(30) Robinson, J. W.; Secker, C.; Weidner, S.; Schlaad, H. Thermoresponsive Poly(N-C3 Glycine)s. Macromolecules 2013, 46 (3), 580–587.
(31) Waley, S. G.; Watson, J. The Kinetics of the Polymerisation of Sarcosine Carbonic Anhydride. Proc. R. Soc. Lond A 1949, 199 (1059), 499–517.
(32) Sisido, M.; Imanishi, Y.; Toshinobu, H. Molecular Weight Distribution of Polysarcosine Obtained by NCA Polymerization. Macromolecular Chemistry and Physics 1977, 178, 3107–3114.
(33) Wu, Y.; Zhou, M.; Chen, K.; Chen, S.; Xiao, X.; Ji, Z.; Zou, J.; Liu, R. Alkali-Metal Hexamethyldisilazide Initiated Polymerization on Alpha-Amino Acid N-Substituted N-Carboxyanhydrides for Facile Polypeptoid Synthesis. Chinese Chemical Letters 2021, 13 (5), 1675–1678.

(34) Xuan, S.; Gupta, S.; Li, X.; Bleuel, M.; Schneider, G. J.; Zhang, D. Synthesis and Characterization of Well-Defined PEGylated Polypeptoids as Protein-Resistant Polymers. Biomacromolecules 2017, 18 (3), 951–964.
(35) Bai, T.; Ling, J. Polymerization Rate Difference of N-Alkyl Glycine NCAs: Steric Hindrance or Not? Biopolymers 2019, 110 (4), e23261.
(36) Fuller, W. D.; Cohen, M. P.; Shabankareh, M.; Blair, R. K.; Goodman, M.; Naider, F. R. Urethane Protected Amino Acid N-Carboxyanhydrides and Their Use in Peptide Synthesis. J Am Chem Soc 1990, 112 (20), 7414–7416.
(37) Becke, A. D. Density-functional Thermochemistry. III. The Role of Exact Exchange. The Journal of Chemical Physics 1993, 98 (7), 5648–5652.
(38) Neese, F.; Wennmohs, F.; Becker, U.; Riplinger, C. The ORCA Quantum Chemistry Program Package. The Journal of Chemical Physics 2020, 152 (22), 224108.
(39) Marenich, A. v; Cramer, C. J.; Truhlar, D. G. Universal Solvation Model Based on Solute Electron Density and on a Continuum Model of the Solvent Defined by the Bulk Dielectric Constant and Atomic Surface Tensions. The Journal of Physical Chemistry B 2009, 113 (18), 6378–6396.
(40) Fetsch, C. Polypeptoide - Synthese Und Charakterisierung. Julius-Maximilians-Universität Würzburg, 2015.
(41) Song, Z.; Tan, Z.; Cheng, J. Recent Advances and Future Perspectives of Synthetic Polypeptides from N-Carboxyanhydrides. Macromolecules 2019, 52 (22), 8521–8539.
(42) Leuchs, H.; Geiger, W. Über Die Anhydride von α-Amino-N-Carbonsäuren Und Die von α-Aminosäuren. Berichte der deutschen chemischen Gesellschaft 1908, 41 (2), 1721–1726.
(43) Leuchs, H.; Manasse, W. Über Die Isomerie Der Carbäthoxyl-Glycyl Glycinester. Berichte der deutschen chemischen Gesellschaft 1907, 40 (3), 3235–3249.
(44) Katchalski, E.; Spitnik, P. Ornithine Anhydride. J Am Chem Soc 1951, 73 (6), 2946–2947.
(45) Ben-Ishai, D.; Katchalski, E. Synthesis of N-Carboxy-α-Amino Acid Anhydrides from N-Carbalkoxy-α-Amino Acids by the Use of Phosphorus Tribromide. J Am Chem Soc 1952, 74 (14), 3688–3689.
(46) Farthing, A. C. 627. Synthetic Polypeptides. Part I. Synthesis of Oxazolid-2 : 5-Diones and a New Reaction of Glycine. J Chem Soc 1950, 3213–3217.
(47) Oya, M.; Katakai, R.; Nakai, H.; Iwakura, Y. A Novel Synthesis of N-Carboxy-α-Amino Acid Anhydride. Chemistry Letters 1973, 2 (11), 1143–1144.
(48) Daly, W. H.; Poché, D. The Preparation of N-Carboxyanhydrides of α-Amino Acids Using Bis(Trichloromethyl)Carbonate. Tetrahedron Letters 1988, 29 (46), 5859–5862.
(49) Kricheldorf, H. R.; Lossow, C. V.; Schwarz, G.; Fritsch, D. Chain Extension and Cyclization of Telechelic Polysarcosines. Macromolecular Chemistry and Physics 2005, 206 (12), 1165–1170.
(50) Guo, L.; Lahasky, S. H.; Ghale, K.; Zhang, D. N -Heterocyclic Carbene-Mediated Zwitterionic Polymerization of N-Substituted N -Carboxyanhydrides toward Poly(α-Peptoid)s: Kinetic, Mechanism, and Architectural Control. J Am Chem Soc 2012, 134 (22), 9163–9171.
(51) Lee, C. U.; Smart, T. P.; Guo, L.; Epps, T. H.; Zhang, D. Synthesis and Characterization of Amphiphilic Cyclic Diblock Copolypeptoids from N-Heterocyclic Carbene-Mediated Zwitterionic Polymerization of N-Substituted N-Carboxyanhydride. Macromolecules 2011, 44 (24), 9574–9585.

(52) Lahasky, S. H.; Serem, W. K.; Guo, L.; Garno, J. C.; Zhang, D. Synthesis and Characterization of Cyclic Brush-Like Polymers by N -Heterocyclic Carbene-Mediated Zwitterionic Polymerization of N -Propargyl N -Carboxyanhydride and the Grafting-to Approach. Macromolecules 2011, 44, 9063–9074.
(53) Brenner, M.; Photaki, I. Zur Herstellung Der Chlorid-hydrochloride Der α-Aminosäuren. Helvetica Chimica Acta 1956, 39 (6), 1525–1528.
(54) Kramer, J. R.; Deming, T. J. General Method for Purification of α-Amino Acid-N-Carboxyanhydrides Using Flash Chromatography. Biomacromolecules 2010, 11 (12), 3668–3672.
(55) Katchalski, E.; Sela, M. Synthesis and Chemical Properties of Poly-α-Amino Acids. Advances in Protein Chemistry 1958, 13 (C), 243–492.
(56) Dorman, L. C.; Shiang, W. R.; Meyers, P. A. Purification of γ-Benzyl and γ-Methyl L-Glutamate N-Carboxyanhydrides by Rephosgenation. Synthetic Communications 1992, 22 (22), 3257–3262.
(57) Ballard, D. G. H.; Bamford, C. H. 77. Reactions of N-Carboxy-α-Amino-Acid Anhydrides Catalysed by Tertiary Bases. J. Chem. Soc. 1956, 0 (0), 381–387.
(58) Liu, J.; Ling, J. DFT Study on Amine-Mediated Ring-Opening Mechanism of α-Amino Acid N -Carboxyanhydride and N-Substituted Glycine N-Carboxyanhydride: Secondary Amine versus Primary Amine. The Journal of Physical Chemistry A 2015, 119 (27), 7070–7074.
(59) Xing, C.; Shi, Z.; Tian, J.; Sun, J.; Li, Z. Charge-Determined LCST/UCST Behavior in Ionic Polypeptoids. Biomacromolecules 2018, 19 (6), 2109–2116.
(60) Hall, H. K. Correlation of the Base Strengths of Amines. J Am Chem Soc 1957, 79 (20), 5441–5444.