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A versatile polypeptoid platform based on *N*-allyl glycine†

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***N*-Allyl glycine *N*-carboxyanhydride (NCA) was synthesized and polymerized by ring opening polymerization under homo- or heterophase conditions to afford well-defined polypeptoids (M_W = 1.5–10.5 kg mol^{-1}, PDI = 1.1–1.4). Poly(*N*-allyl glycine) demonstrated stimuli-responsive behaviour in water and was readily modified *via* thiol–ene photochemistry under experimentally benign conditions.**

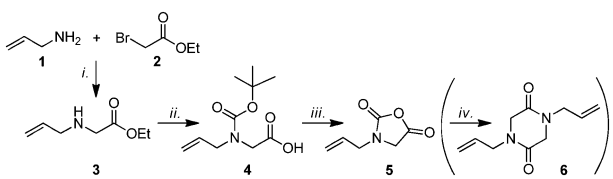
Pseudo-peptides and peptidomimetics,[1] such as β-peptides, peptoids, and also poly(*N*-acyl ethyleneimine)s, have been, and are currently being, investigated for their stimuli-responsiveness, self-assembly, and/or formation of hierarchical structures.[2–4] Investigations are being driven forward by their respective potential in biomedical applications, such as therapeutics (*i.e.* drug delivery systems)[5] and tissue engineering (*i.e.* extracellular matrices).[6] Intuitively, these polymers, constructed with amides in the backbone and/or side chain, allow for an increase in biocompatibility. Particularly, sequence and non-sequence specific *N*-substituted glycine oligomers (*aka* α-peptoids) have been demonstrated to resist enzymatic degradation[7] while maintaining prescribed biological activity.[8] Furthermore, peptoids have been shown to form stable polyproline type I helices[9] and self-assemble.[10,11] Additional application based investigations have included DNA drag tags,[12] nanosheets,[13] cell permeation labels,[14,15] and calcium carbonate mineralization.[16]

A straightforward and efficient submonomer solid-phase synthetic technique was developed[17,18] and improved[19,20] for these peptide regioisomers. More recently, a gram-scale solution-phase synthesis was described, which closely resembles the submonomer approach with repetitive rotovaporizations.[21] A more frequently utilized solution phase synthesis that leads to well-defined polypeptide materials includes the ring opening polymerization (ROP) of α-amino acid *N*-carboxyanhydrides (NCAs).[22] Previously, these techniques were adapted by Kricheldorf *et al.* for *N*-methyl glycine (sarcosine) during their extensive investigations into the active monomer and zwitterion mechanisms of *N*-unsubstituted NCAs.[23–26] Zhang *et al.* described the first bottom up approach of *N*-substituted NCAs that were polymerized *via* an *N*-heterocyclic carbene (NHC).[27,28] Recently, Luxenhofer *et al.* provided a detailed report of a series of peptoid homo- and block copolymers.[29] Due to our interest in "smart" bioinspired, or biohybrid, polymers (in particular glycopolymers), we rationalized that poly(*N*-allyl glycine), as a regioisomer of poly(α-allyl glycine)[30] and a structural isomer of poly[2-(3-butenyl)-2-oxazoline],[31] would be an interesting initial target.

A caveat of our approach includes the post modification of the side chains as opposed to the synthesis of a complex monomer prior to polymerization.[32,33] Although post modifications often suffer from incomplete functionalization and poor repeating unit selectivity, they greatly enable the user to screen a number of hydrophobic, hydrophilic, and binding moieties for increased solubility and receptor interaction from one platform. The advancements in polymer click chemistry (*e.g.* azide–alkyne [3 + 2], (hetero-) Diels–Alder [4 + 2] cycloadditions, thiol–ene/yne additions, *etc.*) have been shown to overcome such problems and facilitate high conversions.[34,35]

The *N*-allyl glycine NCA monomer was synthesized starting from allylamine (**1**) and α-bromo ethylacetate (**2**), as outlined in Scheme 1 (see ESI† for experimental details). Steps i and ii were adapted from a procedure disclosed by Liskamp *et al.*[36] and step iii was modified from a protocol by Zhang *et al.*[27] (Leuchs' method) affording us the *N*-allyl glycine NCA (3-allyloxazolidine-2,5-dione, **5**) in high purity as a colourless oil with an overall yield of 58% (step i → iii) (ESI†). This was a straightforward three-step synthesis that required simple purification techniques such as flash chromatography, liquid–liquid extraction, and vacuum distillation, respectively (ESI†). However, during our early distillation attempts for the crude NCA **5**, we also isolated 1,4-diallylpiperazine-2,5-dione (**6**) with a yield of 31% (step iv). Although cyclic peptides and peptoids have been previously reported,[23] the heat-induced formation of **6** is still unclear.

Polymerizations of **5** (Scheme 2, step (i)) were carried out at concentrations of 1–2 wt% in CH_2Cl_2, *N*,*N*′-dimethylacetamide



Scheme 1 Synthesis of *N*-allyl glycine NCA. (i) NEt_3, THF, 0 °C; (ii) (a) Boc_2O, dioxane, (b) NaOH, MeOH; (iii) PCl_3, CH_2Cl_2, 0 °C; (iv) > 100 °C.

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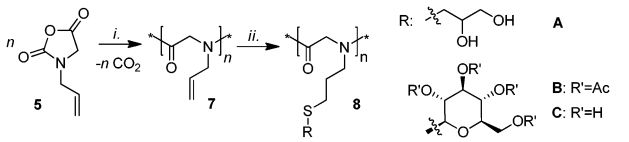


Scheme 2 Synthesis and functionalization of poly(*N*-allyl glycine). (i) (a) $BnNH_2$, r.t., (b) Ac_2O; (ii) R-SH, DMPA, *hν*, r.t.

(DMA), or benzonitrile (PhCN) in sealable flasks (where reduced pressure techniques were employed)[37] and initiated by a 1 M solution of benzyl amine ($BnNH_2$) in *N*-methyl-2-pyrrolidone (NMP; targeted number of repeat units, *n*: $[\mathbf{5}]_0/[BnNH_2]_0$ = 20, 100) at room temperature.[38] Reactions were terminated with acetic anhydride when either consumption of the monomer was determined or no further progress was observed by GC-MS. The molecular characteristics of the isolated polymer samples **7a–c** are summarized in Table 1. In general, the monomer was consumed over 2–5 days for oligomers ($n < 25$) and 3–4 weeks for polymers ($n \sim 50$) in CH_2Cl_2. Further analysis indicated that the monomer was not fully incorporated into the polymer chain ($[\mathbf{5}]_0/[BnNH_2]_0 = 100$) suggesting that degradation occurred in CH_2Cl_2 (data omitted). In contrast, the monomer was found to be stable over the duration for which the polymerizations ran in DMA or PhCN. As expected from earlier reports of Luxenhofer *et al.*,[29] the sample with the highest molecular weight ($\bar{n} \sim 80$) was obtained in PhCN (DMA: $\bar{n} \sim 34$). Due to these results, a polymerization of **5** on a larger scale (5 g) was conducted in PhCN (10 wt%) targeting a polymer with 20 repeating units. After 4 days, the monomer was no longer detectable by GC-MS, the propagating end group was deactivated with acetic anhydride (Ac_2O), and the polymer (**7d**) was isolated by precipitation in petroleum ethers and centrifugation. Gravimetric yield was quantitative and ^{1}H-NMR end group analysis indicated $\bar{n} \sim 18$. In addition, this sample's chemical structure was exemplarily confirmed by MALDI-ToF MS (Fig. 1a: mass of repeat unit, Δm = 97.2 Da ($[C_5H_7NO]^+$ = 97.1 g mol^{-1}); residual mass, r.m. = 149.1 (BnNH/Ac, $C_9H_{11}NO$); $\bar{n} \sim 20$).

We further attempted ROP of the **NCA** under heterophase conditions. A mixture of **5** (0.6 mL) and non-solvent heptanes (30 mL; C_7H_{16}),[39] previously degassed with argon, was initiated with a 1 M solution of $BnNH_2$/NMP ($[\mathbf{5}]_0/[BnNH_2]_0$ = 400) by injecting into (i) a rapid stirring solution, (ii) the layer of

Table 1 Molecular characteristics of poly(*N*-allyl glycine)s obtained under different reaction conditions

Sample	Solvent	$[\mathbf{5}]_0/[BnNH_2]_0$	Reaction time (d)	Yield (%)	n^d	$M_n{}^e$ (kg mol^{-1})	PDI^f	$T_g{}^g$ (°C)
7a	CH_2Cl_2	20	4	72	14	1.5	1.16	—
7b	DMA	100	15	35	34	3.4	1.23	50
7c	PhCN	100	28	59	80	7.9	1.10	54
7d	PhCN	20	4	>99	18	1.9	1.07	55
7e	$C_7H_{16}{}^a$	400	0.5	n/a	98	9.7	1.10	51
7f	$C_7H_{16}{}^b$	400	0.5	n/a	105	10.3	1.33	60
7g	$C_7H_{16}{}^c$	400	0.5	n/a	107	10.5	1.39	60

[a] Heterogeneous phase with initiator added to the high stirring solution. [b] Heterogeneous phase with initiator added to the stagnant heptanes. [c] Heterogeneous phase with initiator added directly into the monomer layer. [d] Average number of repeat units (^{1}H NMR). [e] Number-average molecular weight (^{1}H NMR). [f] Apparent polydispersity index (SEC). [g] Glass transition temperature (DSC) (ESI).

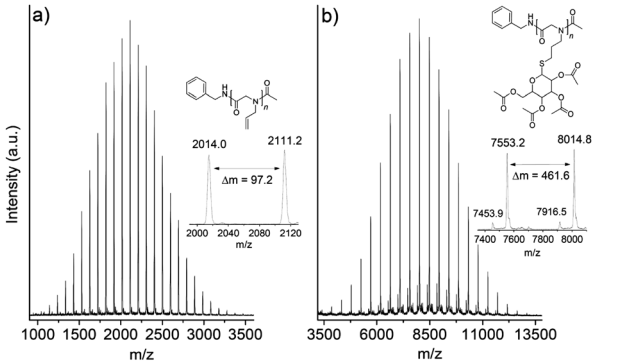


Fig. 1 MALDI-ToF mass spectra (matrix: DCTB, Na^+) of (a) poly(*N*-allyl glycine) **7d** and (b) derived glucosylated polyglycine **8B**.

stagnant heptanes, and (iii) directly into the monomer oil. The reactions were terminated 12 hours later (before full conversion of NCA was reached, GC-MS) with the addition of acetic anhydride following the aforementioned approach. The isolated polymers **7e–g** had similar molecular weights ($\bar{n} \sim 100$, ^{1}H NMR) and PDIs < 1.4 (SEC) (Table 1). Procedure (i) appears to be the best suited to produce high molecular weight polymers with a low PDI (~1.1; **7e**).

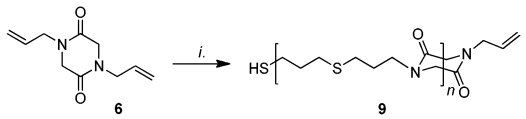
Poly(*N*-allyl glycine)s exhibit glass transition temperatures (T_g) in the range of 50–60 °C (DSC, Table 1) and are thermally stable up to 200–300 °C (thermogravimetric analysis, TGA) (ESI†). In general, the polymers were soluble in organic solvents such as MeCN, PhCN, DMF, DMA, NMP, methanol, ethanol, THF, CH_2Cl_2, and $CHCl_3$ (ESI†). Furthermore, low molecular weight samples ($\bar{n} < 20$) are soluble in water (~ 1% w/w) and show lower critical solution (LCST) behaviour. The cloud point temperature (T_{cp}) of **7d** was 32 °C at 0.1 wt% in water (ESI†).

For conceptual purposes, thiol–ene additions (Scheme 2, step ii) were carried out on poly(*N*-allyl glycine)s **7d** ($\bar{n} \sim 18$) and **7g** ($\bar{n} \sim 107$) with 1-thioglycerol (**A**) (→ samples **8A** and **8A′**, respectively, Table 2). Additionally, **7d** was post modified with 1-thio-β-D-glucose tetraacetate (HS-$GlcAc_4$, **B** → **8B**) and 1-thio-β-D-glucose (HS-Glc, **C** → **8C** and **8C′**). In general, polymers were dissolved at 10% w/w in DMF, or 1% w/w in H_2O (solubility restrictions), with the respective thiol ($[SH]_0/[C{=}C]_0$] ~ 1.2) and stirred for 10 minutes prior to the addition of a photo initiator (2,2-dimethoxy-2-phenyl-acetophenone (DMPA) in DMF; 2-hydroxy-4′-(2-hyroxy-ethoxy)-2-methylpropiophenone (HEMP) in H_2O; < 5 mol%), where the combined mixture was then irradiated with UV light (Hg medium pressure lamp) for 12 or 24 hours, respectively. Modest to quantitative degrees of side arm modifications were readily achieved (Table 2), as suggested by ^{1}H NMR analysis of the products in DMF-d_7 (ESI†). Exemplarily, the

Table 2 Thiol–ene modification of poly(*N*-allyl glycine)s

Sample	Precursor	Thiol	Initiator	Solvent	Reaction time (d)	m^a
8A	**7d**	HS-glycerol	DMPA	DMF	0.5	>0.99
8A′	**7g**	HS-glycerol	DMPA	DMF	1	0.94
8B	**7d**	HS-$GlcAc_4$	DMPA	DMF	0.5	>0.99
8C	**7d**	HS-Glc	HEMP	H_2O	1	>0.99
8C′	**7d**	HS-Glc	None	H_2O	1	0.57

[a] Degree of modification (^{1}H NMR) (ESI).



Scheme 3 Thiol–ene step growth polymerization of **6**. (i) 1,3-propanedithiol, EtOH, *hν*, r.t.

MALDI-ToF mass spectrum of **8B** is shown in Fig. 1b, confirming the expected chemical structure of the glycopolypeptoid, Δm = 461.6 Da ($[C_{19}H_{27}NO_{10}S]^+$ = 461.1 g mol^{-1}). However, the mass spectrum reveals traces of a second, not identified yet, homologous series with a mass difference of either −99.3 or +362.1 Da (compared to the main series).

Exceptionally, these preliminary investigations demonstrated that the glycosylation of **7d**, with HS-Glc, was readily achievable under environmentally benign conditions (*i.e.* in an aqueous solution at room temperature). The reaction can be carried out in the presence (**8C**) or absence (**8C′**) of a photo initiator (HEMP), although the addition rate was much higher in the first case (>99% *vs.* 57% conversion after 12 hours, Table 2).

Finally, we attempted polymerization of **6** *via* thiol–ene and acyclic diene metathesis (ADMET) step growth polymerizations (ESI†).[40] Copolymerization of **6** and 1,3-propanedithiol (Scheme 3) yielded polymer **9** with an apparent molecular weight of $M_n \sim 2.0$ kg mol^{-1}, PDI ∼ 2.2 (SEC). ADMET polymerization of **6** using a 2nd generation Hoveyda–Grubbs catalyst failed, presumably due to a suspected coordination of the amide group to the metal center.[41]

In summary, we described a *de novo* synthesis of poly(*N*-allyl glycine)s and the subsequent characterization revealed a well-defined bio-inspired material ($M_n \sim 1.5$–10.5 kg mol^{-1}, PDI = 1.1–1.4) with good dissolution in organic solvents and modest solubility in water. High degrees of polymerization ($\bar{n} \sim 100$) could be achieved *via* heterophase methodology within relatively short reaction times (12 hours *vs.* weeks). Poly(*N*-allyl glycine)s were modified with thio-glycerol or -glucose by utilizing UV light in combination with photo initiators (DMPA or HEMP) in which the degree of modification of the allyl side chains was found to be ≥ 94%. Notably, we demonstrated that the post modifications of these polymers, with unprotected thio-sugars, are feasible in an aqueous medium.

Additionally, an unexpected dimeric side product of the distillation of *N*-allyl glycine NCA, 1,4-diallylpiperazine-2,5-dione, was explored for thiol–ene and ADMET polymerizations, where the first produced a low molecular weight polymer.

Although there is intrinsic biocompatibility, bioactivity (modification with active groups), and increased proteolytic resistance (*vide supra*), further investigations need to be carried out to verify this potential as a new class of bio-materials.

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