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Solid-phase S-3CR generates N-substituted α-aminonitriles for the synthesis of α-phenyl-α-(1-piperazinyl) substituted amino acids

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Summary. Structurally diverse amino acids were prepared as versatile synthons for combinatorial chemistry. Using an optimized solid-phase synthesis by Strecker-three-component-reaction (S-3CR), two different polymer linker constructs carrying piperazine were investigated. (a) Acrylate derived base-labile linker yielded α-aminonitriles with N-alkylated piperazines via Hofmann elimination after quarternisation with an alkyl halide. The crude product purities were in the range of 54–87%. (b) A urethane type linker yielded α-aminonitriles with the free piperazine nitrogen when cleaved with acid and the product purities were 72–93%. The α-aminonitriles were easily converted to novel N^{ε} – Fmoc-protected α-amino acids with α-(1-piperazinyl) and α-phenyl substituents.

Keywords: Amino acid synthesis – Strecker reaction – Solid-phase synthesis – Combinatorial chemistry – Multicomponent reaction

Introduction

Solid-Phase Organic Synthesis (SPOS) allows parallel operations in combinatorial chemistry to construct libraries of small molecules for drug discovery (Früchtel, 1996; Jung, 1996, 1999). The Strecker-three-component-reaction (S-3CR) is an important de novo method of amino acid synthesis (Strecker, 1850) and variants for synthesizing chiral amino acids have been developed (Göger, 2003). Only a few applications in combinatorial chemistry such as for the development of chiral catalysts were reported (Vachal, 2002). One heterogeneous procedure using alumina support and ultrasound (Hanafusa, 1987) has been described in addition to the more common procedures carried out in liquid phase (Brown, 1997; Ahlbrecht, 1985; Coderc, 1993). Here, we report the SPOS of polymer bound N-substituted benzylic α-aminonitriles (Enders, 2000), which serve as intermediates e.g. in the preparation of collections of novel unnatural α-amino acids for combinatorial syntheses.

Materials and methods

General

Wang resin (capacity: 1.17 mmol/g, 200–400 mesh) was obtained from Rapp Polymere (Germany). ^{1}H-NMR and ^{13}C-NMR: AMX-400 NMR spectrometer (Germany). Electrospray ionization mass (ESI-MS) spectra: Quattro II triple-quadrupole spectrometer from Micromass (UK). High resolution ESI-FT-ICR-MS: Bruker APEX^{TM}II-ESI-FT-ICR-mass spectrometer (Bruker, Germany). Analytical HPLC: Waters instrument using a Grom NucleoSil C_{18} column (18 cm, 5 µm C_{18} 0.3 ml/min, gradient from 90% water (0.1% TFA) to 100% ACN (0.1% TFA)) at 214 nm. Preparative HPLC: Waters instrument (Germany) using a Grom Nucleo Sil C_{18} column (25 cm, 5 µm C_{18}, 10 ml/min), UV detection at 214 nm. HPLC-MS: ESI-Ion Trap MS (Esquire 3000plus from Bruker, Germany) and a HP 1100 ChemStation (Agilent, Germany) with a Purosphere RP-C_{18} column (2-125-4 mm, 4 µm, Merck, Germany) with a gradient from 100% water (0.1% TFA) to 100% ACN (0.1% TFA) at 214 nm. The flow of 1.5 ml/min was reduced by using a T-split (500 µl/min).

General procedure for the S-3CR with 3-(piperazin-1-yl)-acrylate resin (3)

Polymer bound piperazine (**1**, 500 mg, 0.585 mmol) was suspended in DMF (3 ml), different aromatic aldehydes (**2**, 2.38 mmol, 4 eq) and acetone cyanohydrin (500 µl, 5.46 mmol, 10 eq) were added. After stirring at 60 °C for 48 h the resin **3** was washed and dried.

General cleavage protocol

The resin **3** (400 mg) was treated with TFA/DCM (1:3, 4 ml) and agitated for 1 h at room temperature (rt). After collection of the filtrate, the polymer was washed with DCM and the combined filtrates were concentrated in vacuo. The crude products **4** were lyophilised with t-BuOH/water (4:1, 2 ml) and analysed by HPLC and ESI-MS (Table 1).

3-[4-(Cyanophenylmethyl)-piperazin-1-yl]-propionic acid (4-1)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.78–2.67 (m, 6H), 3.28–3.15 (m, 6H), 4.83 (s, 1H), 7.41–7.31 (m, 5H).

3-[4-(1-Cyano-3-phenylpropyl)-piperazin-1-yl]-propionic acid (4-2)

^{1}H-NMR ($CDCl_3$-d_1) δ: 1.95–1.93 (dd, 2H), 2.77–2.62 (m, 8H), 3.40–3.28 (m, 7H), 7.24–7.07 (m, 5H).

3-{4-[Cyano-(3-hydroxyphenyl)-methyl]-piperazin-1-yl}-propionic acid (4-3)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.77–2.66 (m, 6H), 3.30–3.16 (m, 8H), 4.76 (s, 1H), 6.86–6.75 (m, 6H), 7.14 (dd, 1H).

3-{4-[(2-Chlorophenyl)-cyanomethyl]-piperazin-1-yl}-propionic acid (4-4)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.91–2.81 (dd, 4H), 3.29–3.15 (m, 8H), 5.16 (s, 1H), 7.43–7.30 (m, 1H), 7.54–7.50 (m, 3H).

3-{[4-(2-Carboxyethyl)-piperazin-1-yl]-cyanomethyl}-benzoic acid (4-5)

^{1}H-NMR (DMSO-d_6) δ: 2.69–2.63 (ddd, 5H), 3.20–3.14 (dd, 7H), 5.64 (s, 1H), 7.72–7.58 (m, 2H), 8.03–7.98 (m, 2H).

3-{4-[Cyano-(4-trifluormethylphenyl)-methyl]-piperazin-1-yl}-propionic acid (4-6)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.82–2.72 (m, 6H), 3.31–3.22 (m, 6H), 4.95 (s, 1H), 7.66–7.57 (dd, 4H).

3-{4-[Cyano-(3,4,5-trimethoxyphenyl)-methyl]-piperazin-1-yl}-propionic acid (4-7)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.80–2.72 (m, 6H), 3.32–3.20 (m, 6H), 3.78 (s, 3H), 3.81 (s, 6H), 4.75 (s, 1H), 6.64 (s, 2H).

General procedure for the quarternisation reaction with alkyl bromides (6)

The polymer bound α-aminonitrile (**3**, 500 mg, 0.585 mmol) was suspended in DMF (3 ml) and allyl bromide (**5**-**3**, 250 µl, 2.925 mmol, 5 eq) was added. After stirring at rt for 20 h the resin **6** was washed and dried.

Cleavage via Hofmann elimination

The resin (**6**, 500 mg, 0.585 mmol) was suspended in DCM (4 ml) and DIPEA (200 µl) was added. After agitating for 16 h at rt the combined filtrates were collected and evaporated to yield the nitriles **7**, which were analyzed by HPLC and ESI-MS (Table 2).

(4-Allylpiperazin-1-yl)-phenylacetonitrile (7-1)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.86 (br s, 6H), 3.57 (d, J = 7.5 Hz, 4H), 4.74 (s,1H), 5.41 (dd, J = 1.0 Hz, J = 17.0 Hz, 1H), 5.47 (d, J = 10.0 Hz, 1H), 5.96–5.80 (m, 1H), 7.43–7.33 (m, 5H).

2-(4-Allylpiperazin-1-yl)-4-phenylbutyronitrile (7-2)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.09–1.94 (td, 2H), 2.80–2.60 (m, 5H), 2.93–2.86 (m, 3H), 3.32 (s, 1H), 3.41 (t, 2H), 3.59 (d, 2H), 5.52–5.41 (m, 2H), 5.94–5.77 (m, 1H), 7.28–7.09 (m, 5H).

(4-Allylpiperazin-1-yl)-(3-hydroxyphenyl)-acetonitrile (7-3)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.78 (s, 5H), 3.57 (d, J = 10.5 Hz, 2H), 4.72 (s, 1H), 5.50–5.34 (dd, J = 10.0 Hz, 2H), 5.91–5.75 (m, 1H), 6.89–6.77 (m, 3H), 7.28–7.09 (m, 1H).

(4-Allylpiperazin-1-yl)-(2-chlorophenyl)-acetonitrile (7-4)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.87 (s, 5H), 3.57 (d, J = 10.5 Hz, 2H), 5.10 (s, 1H), 5.50–5.37 (dd, 2H), 5.93–5.77 (m, 1H), 7.42–7.26 (m, 3H), 7.55–7.50 (m, 1H).

3-[(4-Allylpiperazin-1-yl)-cyanomethyl]-benzoic acid (7-5)

^{1}H-NMR (DMSO-d_6) δ: 2.67 (br s, 2H), 3.37 (br s, 5H), 3.74 (d, J = 7.5 Hz, 2H), 5.52–5.45 (dd, 2H), 5.67 (s, 1H), 5.96–5.82 (m, 1H), 7.72–7.59 (m, 2H), 8.04–7.98 (m, 2H).

(4-Allylpiperazin-1-yl)-(4-trifluoromethyl-phenyl)-acetonitrile (7-6)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.85 (s, 5H), 3.59 (d, J = 7.5 Hz, 2H), 4.88 (s, 1H), 5.51–5.39 (dd, 2H), 5.94–5.78 (m, 1H), 7.66–7.56 (m, 4H).

(4-Allylpiperazin-1-yl)-(3,4,5-trimethoxyphenyl)-acetonitrile (7-7)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.85 (s, 5H), 3.59 (d, J = 7.5 Hz, 2H), 3.78 (s, 3H), 3.81 (s, 6H), 4.70 (s, 1H), 5.51–5.40 (dd, 2H), 5.96–5.79 (m, 1H), 6.62 (s, 2H).

General procedure for the S-3CR with urethane-linked piperazinyl resin (9)

The resin (**8**, 500 mg, 0.585 mmol) was suspended in DMF (3 ml) and different benzaldehydes (**2**, 2.38 mmol, 4 eq) and acetone cyanohydrin (500 µl, 5.46 mmol, 10 eq) were added. After stirring at 60 °C for 48 h the resin was washed and dried. Cleavage of **10** from the resin as described for **4**, for analysis see Table 3.

Phenyl-piperazin-1-yl-acetonitrile (10-1)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.74 (dd, 4H), 3.18 (dd, 4H), 4.88 (s, 1H), 7.46–7.33 (m, 5H).

(3-Hydroxyphenyl)-piperazin-1-yl-acetonitrile (10-3)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.72 (dd, 4H), 3.09 (dd, 4H), 4.83 (s, 1H), 6.92–6.78 (m, 3H), 7.17 (dd, 1H).

(2-Chlorophenyl)-piperazin-1-yl-acetonitrile (10-4)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.81–2.80 (dd, 4H), 3.20–3.08 (dd, 4H), 5.06 (s, 1H), 7.42–7.26 (m, 3H), 7.54–7.49 (m, 1H).

3-(Cyanopiperazin-1-yl-methyl)-benzoic acid (10-5)

^{1}H-NMR (DMSO-d_6) δ: 2.58–2.63 (m, 2H), 2.82–2.77 (m, 2H), 3.38 (s, 4H), 5.46 (s, 1H), 7.73–7.59 (m, 2H), 8.04–7.98 (m, 2H).

Piperazin-1-yl-(4-trifluoromethylphenyl)-acetonitrile (10-6)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.82–2.67 (m, 4H), 3.26–3.11 (m, 4H), 4.96 (s, 1H), 7.67–7.59 (m, 4H).

Piperazin-1-yl-(3,4,5-trimethoxyphenyl)-acetonitrile (10-7)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.78 (br s, 4H), 3.20 (br s, 4H), 4.72 (s, 1H), 6.64 (s, 2H).

Mixed compound library

3-(Piperazin-1-yl)-acrylate resin (**3**, 250 mg, 0.0293 mmol) and urethane-linked piperazinyl resin (**8**, 250 mg, 0.0293 mmol) were suspended in abs

DMF (3 ml) and a mixture of benzaldehydes (**2-1** 11. 8 µl, **2-3** 14.3 mg, **2-4** 13.2 µl, **2-5** 17.6 mg, **2-6** 11. 8 µl, **2-7** 22.9 mg, each 0.117 mmol, total of 1.2 eq) and acetone cyanohydrin (0.5 ml, 5.46 mmol, 10 eq) was added. After stirring at 60 °C for 48 h the resin was washed and dried. Cleavage of the mixed compound library from the resins was performed according to the general cleavage protocol. The analytical data are summarized in Table 4.

S-3CR with polymer-bound 4-hydroxybenzaldehyde (13)

Wang resin loaded with p-hydroxyaldehyde (**11**, 500 mg, 0.565 mmol) was suspended in DMF (3 ml), morpholine (**12**, 207 µl, 2.38 mmol, 4 eq) and acetone cyanohydrin (500 µl, 5.46 mmol, 10 eq) were added. After stirring at 60 °C for 48 h the resin **13** was washed and dried. Cleavage of products **14** according to the general cleavage protocol. Analytical data see Table 5.

(4-Hydroxyphenyl)-morpholin-4-yl-acetonitrile (14)

^{1}H-NMR ($CDCl_3$-d_1) δ: 2.78–2.67 (m, 6H), 3.28–3.15 (m, 6H), 4.83 (s, 1H), 7.41–7.31 (m, 5H).

Saponification of α-amino nitriles (15)

The α-amino nitriles (**10**) were dissolved in conc HCl (2 ml) and heated to 103 °C for 8 h. After removal of the solvent the crystalline amino acids (**15**) remained. The following amounts of educts were saponified **10-1**: 29 mg (0.144 mmol), **10-3**: 38 mg (0.175 mmol), **10-4**: 62 mg (0.263 mmol), **10-5**: 80 mg (0.326 mmol), **10-6**: 38 mg (0.141 mmol). Analytical data for products **15-1** to **15-16** see Table 6.

Phenylpiperazin-1-yl-acetic acid (15-1)

^{1}H-NMR (D_2O-d_2) δ: 3.24–3.18 (m, 4H), 3.47–3.40 (m, 4H), 4.60 (s, 1H), 7.42–7.33 (m, 5H).

(3-Hydroxyphenyl)-piperazin-1-yl-acetic acid (15-3)

^{1}H-NMR (D_2O-d_2) δ: 3.27–3.19 (m, 2H), 3.51–3.42 (m, 6H), 4.62 (s, 1H), 6.92–6.85 (dd, 3H), 7.28–7.24 (dd, 1H).

(2-Chlorophenyl)-piperazin-1-yl-acetic acid (15-4)

^{1}H-NMR (D_2O-d_2) δ: 3.27–3.24 (m, 2H), 3.50–3.46 (m, 6H), 5.11 (s, 1H), 7.46–7.34 (m, 2H), 7.54–7.51 (m, 2H).

3-(Carboxypiperazin-1-yl-methyl)-benzoic acid (15-5)

^{1}H-NMR (D_2O-d_2) δ: 3.36–3.33 (m, 2H), 3.63–3.50 (m, 6H), 5.05 (s, 1H), 7.62–7.58 (dd, 1H), 7.72 (d, 1H), 8.11–8.08 (dd, 2H).

Piperazin-1-yl-(4-trifluoromethylphenyl)-acetic acid (15-6)

^{1}H-NMR (D_2O-d_2) δ: 3.19–3.15 (m, 2H), 3.46–3.42 (m, 6H), 4.69 (s, 1H), 7.56 (d, 2H), 7.71 (d, 1H).

Fmoc protection of amino acids (16)

Amino acids (**15**, 0.0585 mmol) in acetone/water (2:1, 2 ml), $NaHCO_3$ (10 mg, 0.117 mmol, 2 eq) and Fmoc-OSu (20 mg, 0.0585 mmol, 1 eq) or Fmoc-Cl (15 mg, 0.0585 mmol, 1 eq) were stirred for 16 h rt. After removal of acetone the aqueous solution was extracted with $CHCl_3$ and the organic layers were combined, dried with $MgSO_4$ and the solvent was removed in vacuo. The Fmoc-amino acids **16** were characterized by HPLC-MS, ^{1}H- and ^{13}C-NMR (data not shown).

Results

Strecker-reaction with the amine component on solid-phase

From the different HCN generating reagents we chose acetone cyanohydrin (Kobayashi, 1986; Mori, 1990) because this liquid is easy to handle and upon heating decomposes into acetone and HCN. The resin bound amine (**1**, Fig. 1) was prepared by adding acryloyl chlo-

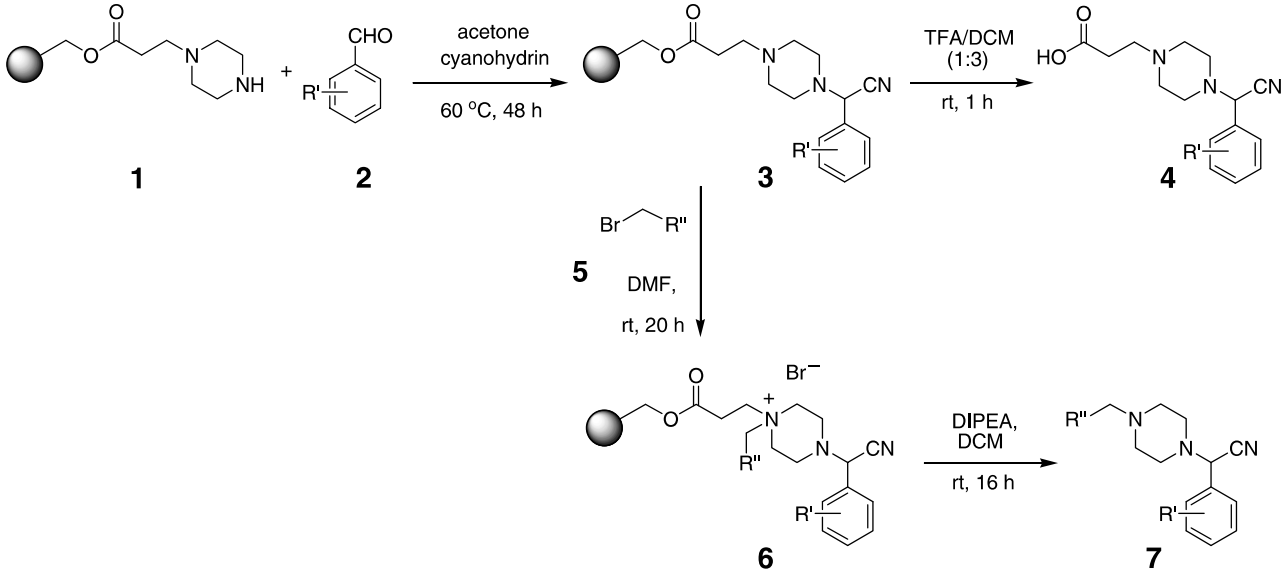

Fig. 1. S-3CR of N$^{\varepsilon}$-substituted α-phenyl-α-(1-piperazinyl)-acetonitriles on solid-phase bound piperazine followed by direct acidic cleavage (**1**) and cleavage of the solid-phase bound quaternized α-aminonitrile (**6**) via Hofmann elimination

Table 1. Variation of the aldehydes (**2**) in the S-3CR for the preparation of α-aminonitriles (**4**)

Aldehydes (**2**) and α-aminonitriles (**4**)		HPLC [min]	HPLC [%]	MS $[M+H]^+$	Yield [%][a]
benzaldehyde	**4-1**	14.12	94	274	89
3-phenylpropionaldehyde	**4-2**	17.37	70	302	97
3-hydroxybenzaldehyde	**4-3**	12.72	88	290	83
2-chlorobenzaldehyde	**4-4**	13.88	83	276	99
3-carboxybenzaldehyde	**4-5**	9.90	93	318	51
4-(trifluoromethyl)-benzaldehyde	**4-6**	18.60	93	342	95
3,4,5-trimethoxybenzaldehyde	**4-7**	15.20	95	364	82
4-hydroxybenzaldehyde	**4-8**	12.42	83	290	–
4-methoxybenzaldehyde	**4-9**	15.57	88	304	–
4-cyanobenzaldehyde	**4-10**	13.17	70	299	–
3-(trifluoromethyl)-benzaldehyde	**4-11**	17.48	90	342	–
3-bromobenzaldehyde	**4-12**	16.52	90	352	–
4-bromobenzaldehyde	**4-13**	16.42	92	352	–
4-chlorobenzaldehyde	**4-14**	16.65	96	308	–
p-tolualdehyde	**4-15**	15.60	90	288	–
4-fluorobenzaldehyde		14.38	86	292	–
3-chloro-4-fluorobenzaldehyde		16.82	82	326	–
3,4-difluorobenzaldehyde		16.37	86	310	–
2,4-dichlorobenzaldehyde		18.13	75	342	–
3-[3-(trifluoromethyl)-phenoxybenzaldehyde	**4-16**	21.92	93	434	–
5-iodovanilline	**4-17**	16.55	89	446	–
4-hydroxy-3-nitrobenzaldehyde	**4-18**	15.05	94	335	–
3-methoxy-4-(4-nitrobenzyloxy)-benzaldehyde	**4-19**	19.92	95	455	–
4-chloro-3-fluorobenzaldehyde		17.72	97	326	–
5-bromo-o-anisaldehyde	**4-20**	16.87	84	382	–

[a] Relative to the initial resin loading, HPLC 214 nm

ride in the presence of diisopropylethylamine (DIPEA) to Wang resin followed by piperazine addition (Brown, 1997). The S-3CR was performed by adding aromatic aldehydes (**2**) and acetone cyanohydrin in the following ratios: resin-bound amine : carbonyl compound : acetone cyanohydrin (1:4:10) in DMF, 48 h at 60 °C; yields 85–99%.

The α-aminonitriles were analyzed by HPLC and ESI-MS after cleavage from the Wang resin. After the establishment of the solid-phase conditions for S-3CR a series of aldehydes, predominantly substituted benzaldehydes, were tested as carbonyl component (**2**) (Table 1).

In general the purities of the isolated crude products were good. However, aliphatic aldehydes gave no products except for 3-phenylpropionaldehyde (**2-2**). Benzaldehydes substituted in position 2 with bulky groups yielded little or no product. Yields of some α-aminonitriles (**4**) from benzaldehydes with electron-withdrawing or electron donating substituents were at least 80% except for 3-{[4-(2-carboxyethyl)-piperazin-1-yl]-cyanomethyl}-benzoic acid (**4-5**). When ketones were used as carbonyl component instead of aldehydes only 5-bromo-2-hydroxyacetophenone gave a yield of 85%.

Hofmann elimination for cleavage of resin bound S-3CR products

To obtain N-alkylated piperazines the Wang resin bound α-aminonitriles (**3**) were subjected to a Hofmann elimination (Kaljuste, 1995). The selective quarternisation of the piperazine nitrogen in position 4 was performed with primary alkyl bromides (**5**) e.g. benzyl bromide (**5-1**), 4-bromobenzyl bromide (**5-2**) or allyl bromide (**5-3**) to give resins **6** (Fig. 1).

Seven representative α-aminonitriles (**3**) were quarternized with allyl bromide (**5-3**) (Table 2). Yields for the α-aminonitriles **7** cleaved via Hofmann elimination were between 53 and 76%. Purities of the crude products were excellent except for the product derived from 2-chlorobenzaldehyde.

Products from quaternisation with 4-bromobenzyl bromide (**5-2**). were obtained with all seven aldehydes, but the purity was lower (Table 2). The products derived from 3-phenylpropionaldehyde (**2-2**) und 3-carboxybenzaldehyde (**2-5**) had a purity of less than 30% (Table 2). The products arising with benzyl bromide (**5-1**) were obtained in slightly better purity (Table 2).

Table 2. α-Aminonitriles (**7**) via Hofmann elimination after alkylation of **3** with allyl bromide (**5-3**), 4-bromobenzyl bromide (**5-2**) and benzyl bromide (**5-1**)

α-Aminonitriles (**7**) from aldehydes (**2**)	Allyl bromide (**5-3**)				4-Bromobenzyl bromide (**5-2**)			Benzyl bromide (**5-1**)		
	HPLC [min]	HPLC [%]	MS $[M+H]^+$	Yield [%][a]	HPLC [min]	HPLC [%]	MS $[M+H]^+$	HPLC [min]	HPLC [%]	MS $[M+H]^+$
7-1	14.95	93	242	64	26.70	63	372	21.40	54	292
7-2	20.05	90	270	60	19.42	26	400	23.98	40	320
7-3	13.50	95	258	76	25.77	58	388	14.82	67	308
7-4	19.95	55	276	63	28.74	67	406	22.26	55	326
7-5	14.25	75	259	53	21.87	28	416	17.98	50	336
7-6	19.25	99	310	58	30.23	70	440	23.77	43	360
7-7	18.45	86	332	53	26.90	61	462	20.17	73	382

[a] Relative to the initial resin loading

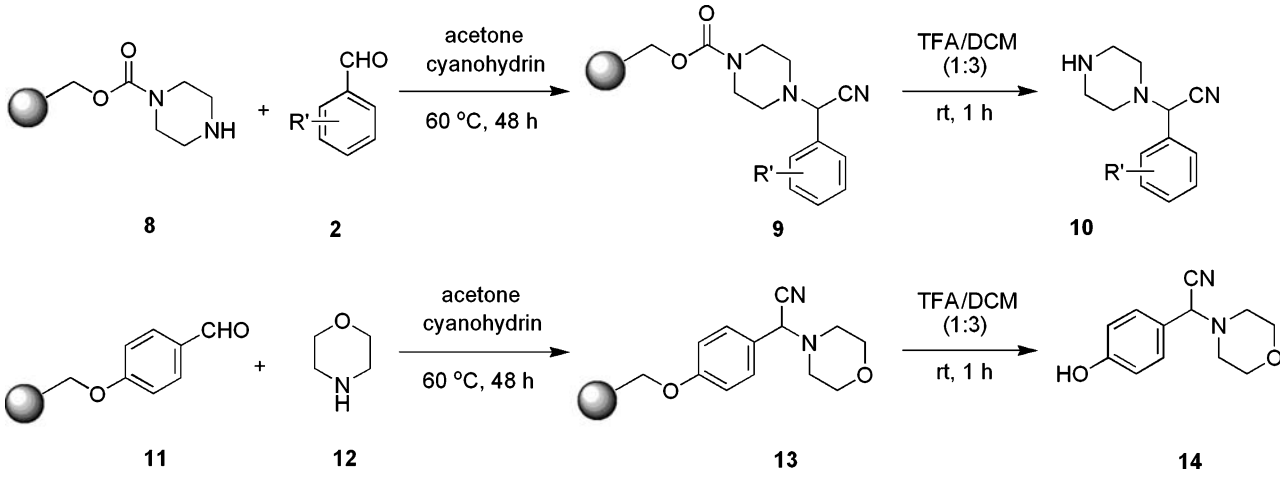

Fig. 2. S-3CR on solid-phase bound piperazine with urethane-linker (**8**) and S-3CR using a solid-phase bound phenolic aldehyde (**11**)

Solid-phase S-3CR using an urethane-linker for the amino component

Further product variations are possible by using a urethane linker for S-3CR synthesis of α-aminonitriles. This linker was prepared by coupling 4-nitrophenyl chloroformate to Wang resin, followed by the reaction of the mixed carbonate with piperazine to give resin **8** (Fig. 2). Reaction conditions were as described (Kaljuste, 1995). Mainly aromatic aldehydes (**2**) were used to obtain a polymer bound α-aminonitrile collection with free nitrogen on the piperazine (**9**).

All aromatic aldehydes gave α-aminonitriles in excellent purities after cleavage from the solid-support and the yields were 40–60% (Table 3). Using 4-(trifluoromethyl)-benzaldehyde (**2-6**) and 3,4,5-trimethoxybenzaldehyde (**2-7**) yields were not satisfying, although the purities were excellent (Table 3).

Table 3. Variation of the aldehyde (**2**) in the solid-phase S-3CR with urethane-linked piperazine (**8**) to yield α-aminonitriles **10**

α-Aminonitriles (**9**) from aldehydes (**2**)	HPLC [min]	HPLC [%]	MS $[M+H]^+$	Yield [%][a]
10-1	13.55	93	202	58
10-2	16.20	28	230	98
10-3	11.32	92	218	40
10-4	22.37	94	236	55
10-5	12.33	86	246	59
10-6	18.78	91	270	31
10-7	14.43	95	292	26
10-8	9.37	77	218	–
10-9	15.13	77	232	–
10-10	17.43	82	236	–
10-15	16.82	83	216	–
10-17	15.57	73	374	–
10-18	8.05	90	252	–
10-19	19.88	91	383	–
10-20	17.85	67	312	–

[a] Relative to the initial resin loading

Table 4. Peak assignment in HPLC-MS and ES-FT-ICR-MS analysis of the mixed compound collection

Educt (**2**)	Linker	HPLC [min]	$[M+H]^+$	Mass calculated	Mass found	Δ Mass[a,b]	δ [ppm][c]	Elemental composition
2-1	acylamino	15.68	274	274.155003	274.156153	0.000115	0.42	$C_{15}H_{20}N_3O_2$
2-3	(**1**)	13.46	290	290.149918	290.149942	0.000024	0.08	$C_{15}H_{20}N_3O_3$
2-4		15.23	308	308.116031	308.116151	0.000120	0.39	$C_{15}H_{19}N_3O_2Cl$
2-5		12.13	318	318.144833	318.144930	0.000097	0.30	$C_{16}H_{20}N_3O_4$
2-6		18.92	342	342.142388	342.142273	0.000115	0.34	$C_{16}H_{19}N_3O_2F_3$
2-7		16.88	364	364.186697	364.186456	0.000241	0.66	$C_{18}H_{26}N_3O_5$
2-1	urethane	14.65	202	202.133874	202.133923	0.000049	0.24	$C_{12}H_{16}N_3$
2-3	(**8**)	12.72	218	218.128789	218.128863	0.000074	0.34	$C_{12}H_{16}N_3O$
2-4		23.73	236	236.094902	236.094886	0.000016	0.07	$C_{12}H_{15}N_3Cl$
2-5		13.15	246	246.123703	246.123653	0.000050	0.20	$C_{13}H_{16}N_3O_2$
2-6		19.28	270	270.121259	270.121191	0.000068	0.25	$C_{13}H_{15}N_3F_3$
2-7		16.28	262	292.165568	292.165503	0.000065	0.22	$C_{15}H_{22}N_3O_3$

[a] Mean value: 0.000135; [b] standard deviation: 0.0002232; [c] mean value: 0.47

Synthesis and analytical investigation of a mixed compound collection

After the establishment and optimization of the S-3CR on solid phase, a small mixed compound collection was synthesized. Wang resins with 3-(piperazin-1-yl)-acrylate (**1**) and with urethane linker (**8**) were mixed in equimolar amounts. A mixture of six different benzaldehydes (**2-1**, **2-3**, **2-4**, **2-5**, **2-6**, **2-7**, total of 1.2 eq) was used for S-3CR as described in Section 3. The resulting mixture of S-3CR products was analysed with HPLC-ESI-MS.

All 12 products were separated by HPLC-ESI-MS. Direct mixture analysis by high resolution ESI-FT-ICR mass spectrometry (Walk, 1999) confirmed the elemental composition of all mixture components (Table 4).

Solid-phase S-3CR with resin bound carbonyl components

An alternative strategy of the solid phase S-3CR is to bind a bifunctional carbonyl component to the solid support. The synthesis was attempted with both, aldehydes and ketones, but only polymer bound aldehydes (**11**) gave successful Strecker reactions (Fig. 2). We used polymer bound 4-hydroxybenzaldehyde attached to Wang resin by Mitsunobu reaction using triphenylphosphine and diethylazodicarboxylate (DEAD) (Hamper, 1996). Pyrrolidine, 1-methylpiperazine und morpholine (**12**) were tested as amine components, but only the latter gave satisfying yields (Table 5).

Saponification of α-amino nitriles and Fmoc-protection of the unnatural α-amino acids

The collection of α-amino nitriles was used for the generation of novel α-amino acids. The common method for the saponification of a nitrile to a carboxylic acid is the

Table 5. S-3CR with polymer-bound phenolic aldehyde (**11**) and cyclic amines to yield α-aminonitriles (**14**)

Amine component in (**14**)	HPLC [min]	HPLC [%]	MS $[M+H]^+$
pyrrolidine	12.25	85	–
1-methylpiperazine	12.40	59	232
morpholine	13.48	84	219

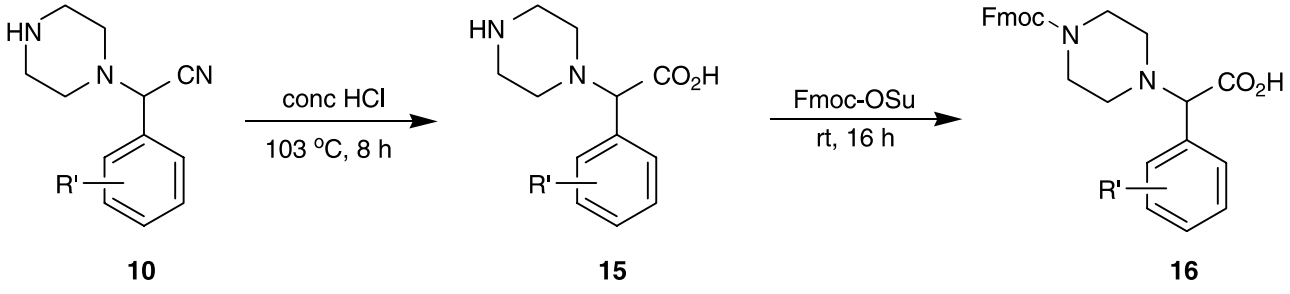

Fig. 3. Saponification of α-phenyl-α-(1-piperazinyl)-phenyl-acetonitriles (**10**) to yield amino acids (**15**) and their Fmoc-protection (**16**)

Table 6. Analytical data of amino acids (**15**) from α-amino nitriles (**10**)

Amino acids **15**	HPLC [Rt] [min]	MS $[M+H]^+$
15-1	0.7	221
15-3	0.7	237
15-4	1.5	255
15-5	0.7	265
15-6	2.4	289
15-8	0.7	237
15-9	0.7	251
15-10	0.7	265
15-11	2.4	289
15-12	2.1	300
15-13	2.3	300
15-14	1.3	255
15-15	0.7	235
15-16	3.5	381

treatment with concentrated mineral acid for several hours. For the preparation of amino acids from the α-aminonitriles bound via the acrylate or urethane modified Wang linker a direct acidic resin saponification may be applied. However, we preferred saponification of the α-aminonitriles (**16**) in solution after their cleavage from the solid-support and their analytical characterisation (Fig. 3).

Various methods for the saponification of nitriles are described using half concentrated or concentrated hydrochloric acid and the temperatures vary from 60 to 103 °C. We found the best turnover with concentrated HCl at 103 °C for 8 h. A representative number of α-amino nitriles (**10**) were treated under the conditions and the resulting α-amino acids (**15**) were analysed by HPLC-MS (Table 6).

The common strategy to produce peptide collections uses the Fmoc protection (Jung, 1996). Therefore the amino group in the piperazine ring was Fmoc protected using 9-fluorenylmethoxycarbonyl-N-hydroxysuccinimide (Fmoc-OSu) or Fmoc-Cl. Phenyl-(piperazin-1-yl)-acetic acid (**15-1**) was treated with Fmoc-OSu (Fernández, 2002) and α-(piperazin-l-yl)-*m*-carboxyphenylacetic acid (**15-5**) with Fmoc-Cl (Mamai, 2001) under basic conditions to yield the corresponding Fmoc-protected α-amino acids (**16**). As examples α-4-(*9H*-fluoren-9-yl-methyloxycarbonyl)piperazin-l-yl) phenylacetic acid (**16-1**) and α-4-(*9H*-fluoren-9-yl-methyloxycarbonyl)piperazin-l-yl)-*m*-carboxyphenylacetic acid (**16-5**) were characterized by NMR and HPLC-MS.

Discussion

α-Amino nitriles are important intermediates in the Strecker synthesis (S-3CR) for the generation of α-amino acids. Via three different attachments of educts we have successfully established the S-3CR synthesis protocols for novel polymer-bound α-aminonitriles substituted with a piperazine ring at the α-amino group. Three different collections of α-aminonitriles with various substitution patterns on the piperazin-1-yl and phenyl rings were synthesised. After optimisation of suitable reaction conditions for the solid-phase S-3CR synthesis a variety of α-amino nitriles were generated by reaction of a polymer bound piperazine with benzaldehydes. Two different linker models were successfully investigated. Firstly, an acrylate linker was used to attach the piperazine moiety to the Wang-resin. Secondly, the quarternisation of the piperazine nitrogen in position 4 of the same Wang-resin with different bromides followed by Hofmann elimination generated α-aminonitriles with N-alkylated piperazine. Thirdly, the usage of an urethane-linker generated α-aminonitriles with a free nitrogen on the piperazine moiety. Saponification of the cleaved α-aminonitriles with strong mineral acid generated a collection of novel α-amino acids, which were Fmoc protected for the use in the peptide mimetic synthesis and in combinatorial chemistry.

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