Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal | View Issue

Cite this: *Org. Biomol. Chem.*, 2014, **12**, 5222

Unusual truncation of *N*-acylated peptoids under acidic conditions†

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Received 24th December 2013,
Accepted 25th April 2014

DOI: 10.1039/c3ob42572j

www.rsc.org/obc

The terminal amino groups of peptoids have often been protected with acetyl groups to improve cell permeability and therapeutic potential, and to prevent the poisoning of the catalysts in organometallic reactions. Interestingly, the unusual truncation of the terminal peptoid unit has sometimes been encountered when the acetylated linear peptoids were treated with a TFA cleavage cocktail. In this study, we systematically investigated the electronic effects of acyl groups on the truncation of *N*-acylated peptoids to rationalize the formation of the deleted peptoids and to establish an appropriate strategy for preventing such undesired truncation.

Introduction

The importance of peptoids has significantly increased since they were first reported by Zuckermann *et al.* in 1992.[1] Peptoids are the structural isomers of peptides with the alkyl substitution at the amide nitrogen instead of the α-carbon of peptides. Peptoids are endowed with diverse structural and functional features, including the ability to fold into secondary structures,[2] assemble into discrete multimers,[3] and act as molecular transporters,[4] nanomaterials,[5] *etc.* Interestingly, in many cases, peptoids are even more advantageous than the corresponding peptides due to their easy chemical synthesis,[6] improved cell permeability,[7] proteolytic resistance,[8] and tolerance towards high salt concentration, organic solvents, and extreme pH. Thus, peptoids have emerged as an attractive class of peptidomimetics in chemical biology, molecular biophysics, drug development, and medical diagnostics.[9]

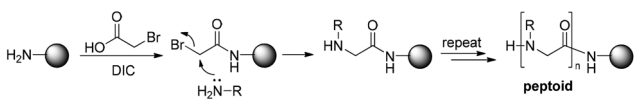
When peptoids have been used as chemical tools in biological application studies, the terminal amino group has often been protected with an acetyl group. The protection of the N-terminal group makes peptoids more hydrophobic, which is known to improve cell permeability, therapeutic potential, *etc.*[10] In addition, we recently reported the facile solid-phase synthesis of cyclic peptoids using ring-closing metathesis (RCM). In the case of the solid-phase RCM, the terminal amino functional groups of the peptoids should be protected with acetyl or Boc groups prior to the RCM step to prevent the poisoning of the Ru catalyst.[11] Interestingly, the unusual truncation of the terminal peptoid unit has sometimes been encountered when the acetylated linear peptoids were treated with a trifluoroacetic acid (TFA) cleavage cocktail (unpublished results). Therefore, it is important to investigate the factors that govern this unusual truncation.

We report herein systematic studies on the scission of *N*-acylated peptoids, which could rationalize the formation of the deleted peptoids and also establish an appropriate strategy to minimize such undesired truncation. We envisioned that the electronic effects might play a very important role in inducing site-specific cleavage leading to the generation of unwarranted peptoids, trimmed at the N-terminal peptoid unit. To this end, we prepared model peptoids with different chain lengths and side chains under different cleavage conditions, and introduced several acyl groups to ascertain the electronic effects.

Results and discussion

Unusual truncation of *N*-acetylated peptoids

Generally, peptoids can be easily synthesized by a solid-phase submonomer protocol,[1] where iterative reactions of acylation with bromoacetic acid followed by nucleophilic displacement of bromine with suitable primary amines provide peptoids with the desired length and sequence (Scheme 1). Microwave-



Scheme 1 Solid-phase synthesis of peptoids.

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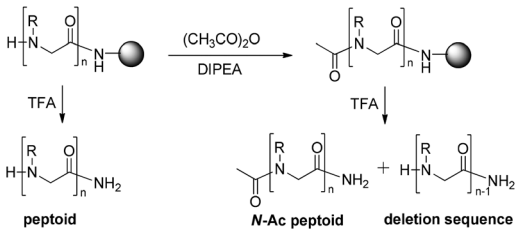
† Electronic supplementary information (ESI) available: MALDI-TOF spectra and RP-HPLC chromatograms. See DOI: 10.1039/c3ob42572j



Scheme 2 Truncation of *N*-acetylated peptoids.

assisted solid-phase synthesis of peptoids has also been well developed.[12]

The terminal amino functional group of peptoids can be readily protected by the treatment of acetic anhydride or acetyl chloride in the presence of *N*,*N*-diisopropylethylamine (DIPEA) to give *N*-acetylated peptoids. However, when *N*-acetylated peptoids are released from the resin by a TFA cleavage cocktail, the truncated products are often detected (Scheme 2). This kind of truncation has never been detected with unprotected peptoids. There have been several reports on the fragmentation of the terminal peptide unit when *N*-acetylated peptides contained *N*-methylamino acid at the terminal position.[13] However, no systematic study has explored this phenomenon in the field of peptoid chemistry. Therefore, the present work has been directed to shed light on the underlying mechanism and to control the formation of the desired *N*-acylated peptoids.

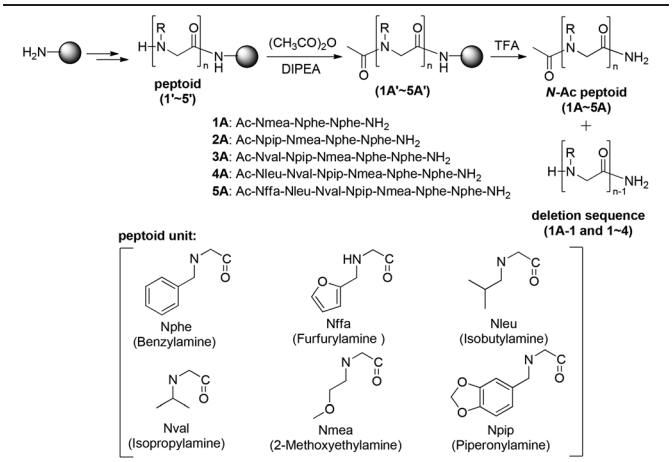
Susceptibility of *N*-acetylated peptoids to TFA-assisted truncation

First, in order to examine the susceptibility of peptoids to TFA-assisted truncation, several model peptoids from a trimer to a heptamer, containing various side chains at the terminal positions, were synthesized on Rink amide resin using the aforementioned procedure and treated with acetic anhydride and DIPEA to provide the acetylated peptoids. Each acetylated peptoid was released from the resin using a TFA cleavage cocktail (Table 1). Regardless of the identity of the alkyl groups at the terminal positions and the length of peptoids, significant amounts of acetylated peptoids were cleaved to give the truncated products upon treatment with 92% TFA in most cases. Similar truncations were detected even with a 50% TFA cleavage cocktail. Furthermore, it was interesting to note that the acetylated pentamer peptoid containing the isopropyl group at the terminal peptoid unit was completely truncated to give the deletion product with 92% TFA or 50% TFA.

Mechanistic aspects

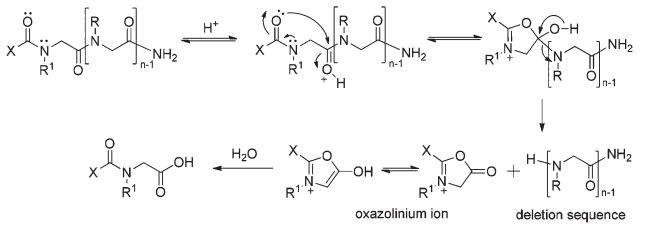
Based on these observations, the involvement of the oxazolinium ion intermediate during the truncation of *N*-acylated peptoids can be proposed (Scheme 3). The electron pair over the terminal amino group shifts towards the carbonyl group of the acyl moiety, facilitating the attack of the carbonyl oxygen at the carbonyl carbon of the adjacent amide linkage to trigger the formation of a five-membered oxazolinium ion intermediate leaving behind the truncated peptoid. The tautomerization of the intermediate oxazolinium ion to the stable aromatic ring may also favor the C–N bond cleavage. The oxazolinium intermediate then tautomerizes to form the stable aromatic ring. Based on the proposed mechanism, it could be conjectured that the electronic effects imparted by both *N*-alkyl and acyl groups might crucially influence the final outcome. We focused on the effects of acyl groups in this study because many primary alkyl amines can be used for peptoid synthesis. If electron-withdrawing acyl groups were introduced, a reduced electron availability or nucleophilicity on the terminal carbonyl

Table 1 TFA-assisted cleavage of *N*-acetylated model peptoids



Entry	Peptoid	Cleavage conditions[a]	Product[b] (%)	
			N-Ac peptoid	Deletion sequence
1	**1A′**	A	**1A** (67.0)	**1A-1** (33.0)
2		B	**1A** (70.7)	**1A-1** (29.3)
3	**2A′**	A	**2A** (80.9)	**1** (19.1)
4		B	**2A** (84.6)	**1** (15.4)
5	**3A′**	A	**3A** (0)	**2** (100)
6		B	**3A** (0)	**2** (100)
7	**4A′**	A	**4A** (74.3)	**3** (25.7)
8		B	**4A** (82.7)	**3** (17.3)
9	**5A′**	A	**5A** (93.8)	**4** (6.2)
10		B	**5A** (94.5)	**4** (5.5)

[a] Conditions A: 92% TFA–3% triisopropylsilane (TIS)–5% H_2O, 1 h; B: 50% TFA–3% TIS–5% H_2O–42% CH_2Cl_2, 1 h. [b] Relative yields of *N*-Ac peptoids and deletion sequences were determined by HPLC analysis.



Scheme 3 Plausible mechanism for the truncation of *N*-acylated peptoids.

oxygen would be expected to retard or prevent the formation of a five-membered oxazolinium intermediate and hence prevent the scission on N-terminal acylated peptoids.

Electronic effects of *N*-acyl groups against TFA-assisted truncation

In order to verify our assumption, we examined the electronic effects based on the identity of the acyl groups. The sequence of the acetylated pentamer peptoid which afforded the truncated product exclusively, was chosen for this study. Considering the electronic effect of acyl groups, the propionyl (Pr), pivaloyl (Piv), benzoyl (Bz), 4-nitrobenzoyl (4NBz) and trifluoroacetyl (TFAc) acyl groups together with the acetyl (Ac) group were employed. The protection of the free terminal amino functionality was accomplished using the corresponding acid chlorides or anhydrides in the presence of DIPEA. Particularly, the introduction of the trifluoroacetyl group required a change of solvent from DMF to THF because of the high electron withdrawing nature of the trifluoroacetyl group, which could readily react with DMF to form a complex.[14] Similarly to *N*-acetylated peptoids, peptoids protected with *N*-Pr, and *N*-Piv groups underwent complete truncation upon treatment with 92% TFA for 1 h to produce the deletion sequence (**2**) (Table 2). However, when the *N*-Bz peptoid was treated with 92% and 50% TFA for 1 h, the desired *N*-Bz peptoid (**3D**) was partially produced in 17.5 and 32.5% yields, respectively. In addition, the *N*-4NBz peptoid where *N*-4NBz should act as an electron-withdrawing group was treated with 92% TFA for 1 h to give the desired *N*-4NBz peptoid (**3E**) and the deletion sequence (**2**) in 96.1% and 3.9% yields, respectively. When the *N*-4NBz peptoid was treated with 92% TFA for 3 h, it was sufficiently stable to be produced in 89.2% yield. Furthermore, the *N*-TFAc peptoid containing a strong electron-withdrawing group was not truncated by the treatment of 92% TFA even after 3 h. Fig. 1 shows the overlay of HPLC chromatograms of *N*-Ac, *N*-4NBz and *N*-TFAc peptoids, revealing the obvious differences.

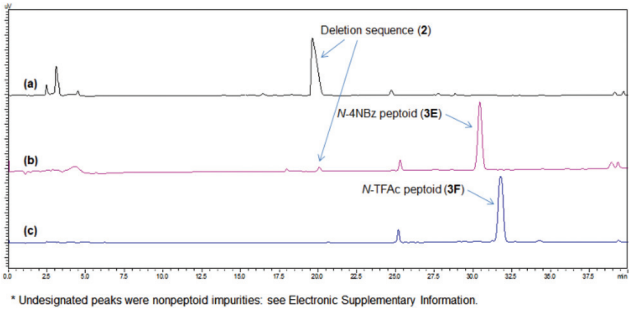
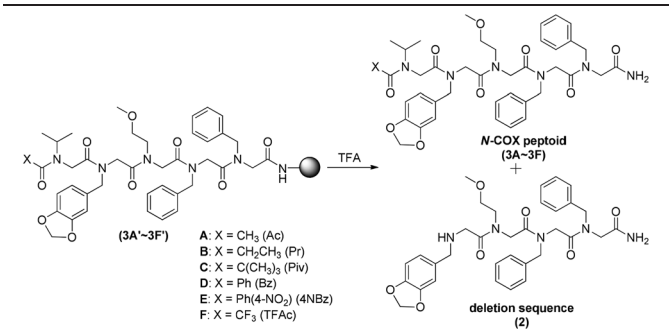


Fig. 1 Overlay of HPLC chromatograms of (a) *N*-Ac (**3A**), (b) *N*-4NBz (**3E**), and (c) *N*-TFAc (**3F**) peptoids.

These results clearly indicate that the reduction of electron density on the carbonyl oxygen by installing electron-withdrawing groups reduced or prevented the scission of the amide bond. All of these observations corroborate our assumptions and advocate the use of an acyl functional group for the protection of N-terminal peptoids. The truncation of *N*-acylated peptoids could be effectively arrested by employing electron-withdrawing groups such as 4NBz and TFAc. Moreover, the use of TFAc was deemed more beneficial especially considering the ubiquitous presence of the trifluoromethyl group as an important moiety in many therapeutic agents.[15]

Table 2 TFA-assisted cleavage of various *N*-acylated peptoids



Entry	Acyl group	Cleavage conditions[a]	Product[b] (%) *N*-COX peptoid	Deletion sequence
1	Ac	A	**3A** (0)	**2** (100)
2		B	**3A** (0)	**2** (100)
3	Pr	A	**3B** (0)	**2** (100)
4		B	**3B** (0)	**2** (100)
5	Piv	A	**3C** (0)	**2** (100)
6		B	**3C** (0)	**2** (100)
7	Bz	A	**3D** (17.5)	**2** (82.5)
8		B	**3D** (32.5)	**2** (67.5)
9	4-NBz	A	**3E** (96.1/89.2[c])	**2** (3.9/10.8[c])
10		B	**3E** (96.4/92.7[c])	**2** (3.6/7.3[c])
11	TFAc	A	**3F** (100/100[c])	**2** (0/0[c])
12		B	**3F** (100/100[c])	**2** (0/0[c])

[a] Conditions A: 92% TFA–3% triisopropylsilane (TIS)–5% H_2O, 1 h; B: 50% TFA–3% TIS–5% H_2O–42% CH_2Cl_2, 1 h. [b] Relative yields of the *N*-COX peptoids and deletion sequences were determined by HPLC analysis. [c] Cleavage time: 3 h.

Conclusions

In conclusion, *N*-acylated peptoids could undergo truncation at the terminal peptoid unit under acidic cleavage conditions to form the deletion sequences. In order to understand and arrest this unusual truncation, systematic studies were carried out using peptoids with different alkyl side chains and different acyl groups. The formation of deletion sequences was reduced or prevented by simply employing electron-withdrawing acyl groups such as 4-nitrobenzoyl (4NBz) and trifluoroacetyl (TFAc) groups based on a plausible mechanism. This study establishes a mechanistic and electronic understanding for the truncation of *N*-acylated peptoids to rationalize the formation of the deleted peptoids and provides an efficient and useful means to prepare N-terminal acylated peptoids without encountering undesired deletion products.

Experimental

General methods

Chemical reagents were purchased from commercial sources and used without further purification unless noted otherwise. The Rink Amide AM resins were purchased from Novabiochem. Reverse-phase HPLC experiments were conducted through an ACE 5 C18-HL (250 × 4.6 mm) reverse phase column on a Shimadzu binary HPLC system equipped with a UV-visible detector at 220 nm. The typical flow rate for the analytical HPLC was 1 mL min^{-1}. MALDI-TOF and MALDI-TOF/TOF MS were performed on an Axima Performance mass spectrometer (Shimadzu) with α-cyano-4-hydroxycinnamic acid as a matrix. The peptoids were synthesized in an incubator shaker (JEIO TECH, model SI-600R) or in a microwave oven (Daewoo, model KR-B200R). The microwave reactions were performed at a power of 100 W for peptoid synthesis.

General procedure for peptoid synthesis

The peptoids were synthesized on Rink amide AM resins by the conventional submonomer strategy.[12] Peptoid syntheses were performed in 25 mL standard glass peptide synthesis vessels. The resins were swelled in DMF at 25 °C for 1–2 h. Then DMF was drained, and the beads were incubated with 20% piperidine in DMF for 30 min and washed thoroughly with DMF (8 × 3 mL). The beads were treated with 2 M bromoacetic acid (1.0–1.5 mL) and 3.2 M DIC (1–1.5 mL) and irradiated in a microwave oven (100 W) for 3 × 12 s with shaking for 30 s after each pulse. The beads were thoroughly washed with DMF (8 × 3 mL) and then treated with primary amines (2 M, 2 mL) in DMF in a microwave oven (100 W) for 3 × 12 s with shaking for 30 s after each pulse. In this case, benzylamine, furfurylamine, isobutylamine, isopropylamine, 2-methoxyethylamine, and piperonylamine were used. Both acylation and displacement were successively repeated to form the desired peptoid sequences.

General procedure for the acylation of the N-terminal of the peptoids

For the synthesis of the *N*-acetylated peptoids, the resins which contained the desired peptoid sequences were treated with acetic anhydride (10 eq.) and *N*,*N*-diisopropylethylamine (DIPEA) (10 eq.) in DMF for 3 h. In order to introduce propionyl (Pr), pivaloyl (Piv), benzoyl (Bz), and 4-nitrobenzoyl (4NBz) at the N-terminal of the peptoids, the resins which contained the desired peptoid sequences were treated with propionyl chloride (30 eq.), pivaloyl chloride (30 eq.), benzoyl chloride (30 eq.), and 4-nitrobenzoyl chloride (30 eq.) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) (30 eq.) in DMF at 40 °C for 3 h, respectively. A trifluoroacetyl (TFAc) group was introduced at the N-terminal of the peptoids on the resins by treatment with trifluoroacetic anhydride (10 eq.), *N*,*N*-diisopropylethylamine (DIPEA) (10 eq.), and 4-dimethylaminopyridine (DMAP) (5 eq.) in THF at room temperature overnight.

Table 3 HPLC retention times and MALDI-TOF data for the peptoids

Entry	Peptoid	HPLC retention time[a] (min)	MALDI-TOF (*m*/*z*) Observed	Calculated
1	**1**	13.8	427.2	427.2 (M + H)
2	**1A**	18.0	491.4	491.2 (M + Na)
3	**1A-1**	10.4	312.4	312.1 (M + H)
4	**2**	19.5	618.2	618.3 (M + H)
5	**2A**	23.2	682.3	682.3 (M + Na)
6	**3**	19.9	717.8	717.4 (M + H)
7	**4**	22.9	830.5	830.4 (M + H)
8	**4A**	26.9	895.0	894.5 (M + Na)
9	**5**	21.0	967.9	967.5 (M + H)
10	**5A**	29.2	1031.4	1031.5 (M + Na)
11	**3D**	29.1	843.9	843.4 (M + Na)
12	**3E**	30.5	888.9	888.4 (M + Na)
13	**3F**	31.7	835.5	835.3 (M + Na)

[a] HPLC conditions: 20% ACN–H_2O to 80% ACN–H_2O with 0.05% TFA for 40 min.

General procedure for the TFA-assisted cleavage and reverse-phase HPLC

The peptoid-tethered resin which was thoroughly washed with DCM was suspended in a cleavage cocktail (condition A: 92% TFA–3% TIS–5% H_2O; condition B: 50% TFA–3% TIS–5% H_2O–42% DCM) for 1–3 h. After the cleavage solution was removed by blowing with N_2 gas, 50% aq. acetonitrile was added and freeze-dried. The mixture was dissolved in 50% aq. acetonitrile containing 0.05% TFA and then filtered through a 0.2 μm PTFE filter tip, and the obtained solution was directly used for the HPLC and MALDI-TOF analyses. The reverse-phase HPLC experiments were conducted through a C18 reverse-phase column on a Shimadzu binary HPLC system equipped with a UV-visible detector at 220 nm. In all cases, the typical flow rate for the analytical HPLC was 1 mL min^{-1} and the gradient elution was gradually changed from 20% ACN–H_2O to 80% ACN–H_2O with 0.05% TFA for 40 min. The relative yields were determined by HPLC analysis based on the integration of each peak. Table 3 shows the HPLC retention times and MALDI-TOF data for the peptoids which were synthesized or produced in this work.

Abbreviations

ACN	Acetonitrile
Ac	Acetyl
Pr	Propionyl
Piv	Pivaloyl
Bz	Benzoyl
4NBz	4-Nitrobenzoyl
TFAc	Trifluoroacetyl
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DCM	Methylene chloride
THF	Tetrahydrofuran

TFA	Trifluoroacetic acid
TIS	Triisopropylsilane
DIC	*N*,*N*′-Diisopropylcarbodiimide
HPLC	High-performance liquid chromatography
MALDI-TOF	Matrix-assisted laser desorption/ionization-time of flight.

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2012R1A1A2006417).

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