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Synthesis of a Non-Peptide Analogue of omega-Conotoxin MVIIA

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Abstract

An efficient synthesis of an alkylphenyl ether based peptidomimetic is described. The compound mimics three key amino acids of omega-conotoxin MVIIA from the cone shell *Conus magus* and may provide an entry to the design of low molecular weight antagonists of N-type neuronal calcium channels.

Keywords: Amidines; Oxadiazoles; Peptide analogues; Phenols; N-type neuronal calcium channels.

ω-Conotoxin MVIIA from the venom of the cone shell *Conus magus* [1,2] is one of the most promising new drugs for the treatment of severe neuropathic pain [3,4]. Its analgesic effect has its roots in a highly selective modulation of N-type neuronal calcium channels. Also known as 'SNX-111' and 'Ziconotide®', it is more potent than morphine and chronic administration does not elicit tolerance or lead to addiction. However, total synthesis of conotoxins is expensive and time-consuming and the toxin cannot be administered orally. These drawbacks initiated our research in the area of low molecular weight conotoxin analogues. Our synthetic strategy follows the 'dendroid approach' to peptidomimetics [5].

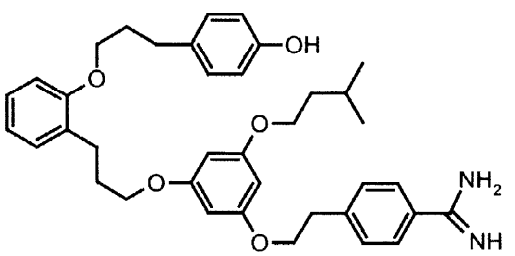

Figure 1. ω-Conotoxin MVIIA analogue **1**.

This approach requires the attachment of side-chain mimetics of the key amino acids to a dendritic backbone. Adjustment of the backbone structure enables the side-chain mimetics to occupy the same positions as the side-chains in the protein. A special feature of dendroid-type peptidomimetics is flexibility of these molecules as a whole. This is in contrast to rigid

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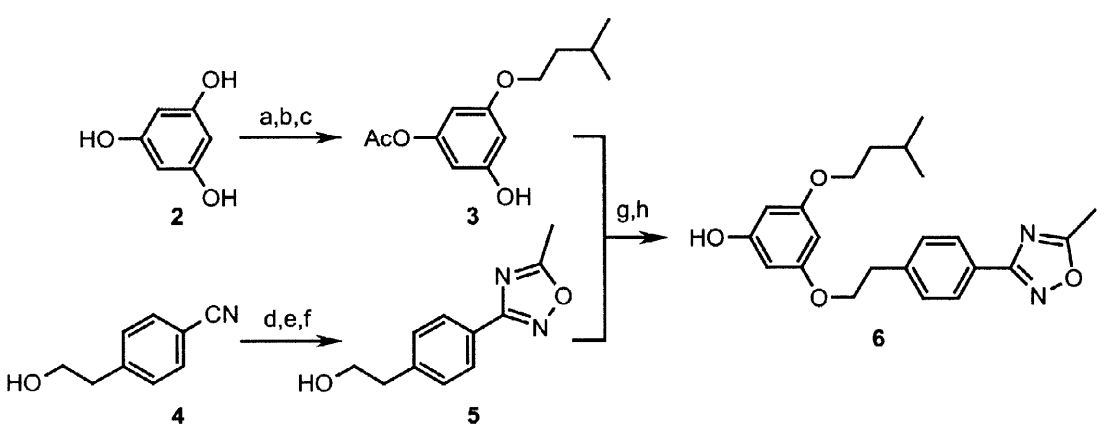
molecules, as the dendroid motif allows each functional group to find the preferred conformation for receptor binding independently. Hence these molecules are preorganised to search global minima, rather than just local minima. In this letter we report the first synthesis of a dendroid type analogue of ω-conotoxin MVIIA.

The key amino acids of the peptide for binding to the N-type calcium channel have been determined to be Lys-2, Arg-10, Leu-11, Tyr-13 and Arg-21 [6]. Analogue **1** (Figure 1) covers the three central amino acids Nos. 10, 11 and 13. For our non-peptide analogue, the side-chain of arginine was replaced by a benzamidino group, tyrosine is mimicked by a *para*-substituted phenol and leucine by an isopentyl residue. The backbone structure has been derived from a 3D structure of ω-conotoxin MVIIA available from the Protein Data Bank [7]. The aryl ether linkages were introduced by high-yielding etherifications under mild conditions following Williamson and Mitsunobu protocols.

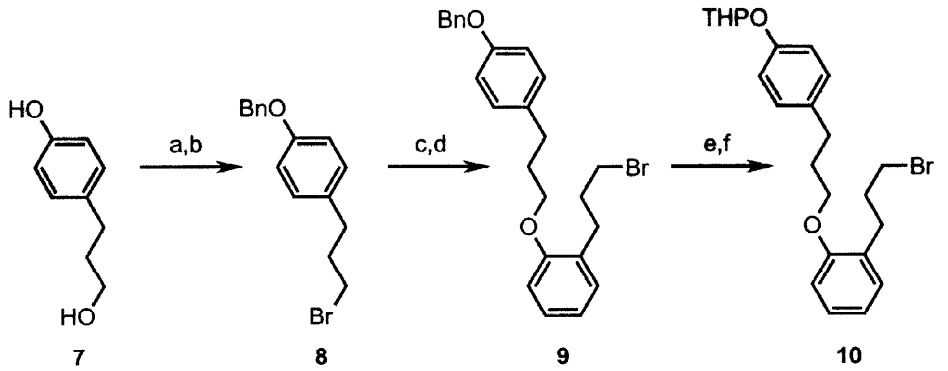
In accordance with a convergent synthetic approach, two main building blocks **6** and **10** were prepared as outlined in schemes 1 and 2. Phloroglucinol **2** (Scheme 1) was chosen as a core unit due to its high reactivity in Williamson etherifications. Alkylation of unprotected **2** led to mixtures of C- and O-alkylated derivatives. To avoid C-alkylation, **2** was converted into its triacetate [8]. Monoalkylation of the triacetate with 1-bromo-3-methylbutane, followed by monodeacetylation yielded phenol **3**. Addition of hydroxylamine to the nitrile **4**, cyclisation of the resulting amidoxime with acetic anhydride and subsequent deacetylation of the alcohol function gave the oxadiazole **5**. The oxadiazole moiety is the benzamidine precursor of choice because it is sufficiently stable to be carried through the entire sequence, but can easily be cleaved by catalytic hydrogenation [9]. **3** and **5** were then combined in a Mitsunobu coupling to give, after deacetylation, phenol **6** in 66% yield.



Scheme 1. *Reagents and conditions.* a) AcOCl / Pyr-CH_2Cl_2 / 0°C-RT / 30 min, 65%; b) 1-Bromo-3-methylbutane / NaH / H_2O / DMF / 0°C-RT / 2.5 h, 47%; c) NaOMe / MeOH / RT / 30 min, 74%; d) NH_2OH / MeOH / RT / 24 h, 57%; e) Ac_2O / 120°C / 4 h, 82%; f) NaOMe / MeOH / RT / 30 min, 94%; g) PPh_3 / DIAD / THF / 0°C-RT / 3 h; h) NaOMe / MeOH / RT / 30 min, 66% over two steps.

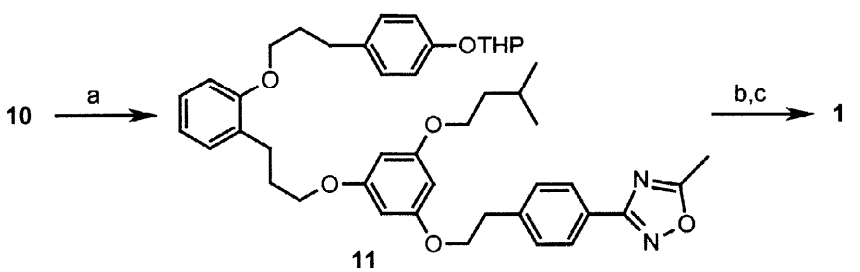
Synthesis of bromide **10** started from diol **7** (Scheme 2) . Protection of the phenolic hydroxyl group as its benzyl ether and bromination of the aliphatic hydroxyl group gave **8**. Williamson etherification of **8** with 3-(2-hydroxyphenyl)-1-propanol and subsequent bromination afforded **9**. Initial attempts to synthesise target compound **1** by reaction of **6** and **9** under Williamson conditions followed by simultaneous debenzylation and cleavage of the

oxadiazole by palladium catalysed hydrogenation failed due to decomposition of the amidine. The benzyl group in **9** was therefore replaced with a tetrahydropyranyl (THP) ether. Etherification of **10** with **6** afforded **11** in 78% yield.



Scheme 2. *Reagents and conditions.* a) BnBr / NaH / DMF / 0°C-RT / 3 h, 70%; b) CBr_4 / PPh_3 / THF / 0°C-RT / 15 h, 93%; c) 3-(2-Hydroxyphenyl)-1-propanol / NaH / DMF / 0°C-RT / 2 h, 82%; d) CBr_4 / PPh_3 / THF / 0°C-RT / 15 h, 91%; e) Pd-C / 1 bar H_2 / EtOAc / RT / 3.5 h, 82%; f) 3,4-Dihydro-2*H*-pyran / PPTS / CH_2Cl_2 / 0°C-RT / 1 h, 86%.

Deprotection of **11** was now accomplished in two steps. Stirring with Dowex-H^+ in methanol-toluene removed the THP group. Subsequent hydrogenation over Raney-nickel resulted in a smooth cleavage of the oxadiazole, giving the acetylamidine. Spontaneous hydrolysis of the latter gave, after flash chromatography, the desired peptide analogue **1** as its acetic acid salt in 78% yield. To prove the structure of **1**, the ^1H and ^{13}C NMR spectra were completely assigned with the aid of COSY, HSQC and HMQC experiments (400 MHz, $CDCl_3$).



Scheme 3. *Reagents and conditions.* a) **5** / NaH / DMF / 0°C-RT / 2 h, 78%; b) Dowex 50WX2-400 / MeOH-PhMe 5:1 / RT / 30 min, 89%; c) Ra-Ni / 4 bar H_2 / EtOH / RT / 5 h, then EtOH-AcOH-H_2O 20:1:1 12h, 78%.

Despite its striking simplicity and mildness, this method for the synthesis of benzamidines is not very widespread in the chemical literature. The work recently reported by Gante [10] and the results presented here should encourage further applications of this methodology.

In summary, we have described an efficient synthesis of a peptide analogue from inexpensive commercial starting materials. All three functional groups, i. e. the benzamidine, the phenol and the isopentyl group, are attached to the dendroid motif by ether linkages and thus may be easily interchanged. This versatility is essential for a successful interplay of molecular modelling, organic synthesis and biological screening. The biological evaluation of conotoxin analogue **1** is in progress.

Selected data. 1xAcOH: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (d, J = 6.6 Hz, 6 H, $CH(C\underline{H}_3)_2$), 1.62 (m, 2 H, $C\underline{H}_2$-CH), 1.79 (m, 1 H, C<u>H</u>), 1.86 (s, 3 H, $C\underline{H}_3COO^-$), 2.00 (m, 2 H, $C\underline{H}_2$), 2.06 (m, 2 H, $C\underline{H}_2$), 2.67 (t, J = 7.5 Hz, 2 H, $C\underline{H}_2$), 2.78 (t, J = 7.5 Hz, 2 H, $C\underline{H}_2$), 3.08 (t, J = 6.1 Hz, 2 H, $C\underline{H}_2$), 3.89 (t, J = 6.7 Hz, 2 H, $C\underline{H}_2$), 3.93 (m, 4 H, $C\underline{H}_2$), 4.08 (t, J = 6.2 Hz, 2 H, $C\underline{H}_2$), 5.98-6.08 (m, 3 H, arom.), 6.66-7.70 (m, 12 H, arom.). ^{13}C NMR (100 MHz, $CDCl_3$) δ 179 ($CH_3\underline{C}OO^-$), 167 ($\underline{C}(NH_2)_2$), 161 (3 C), 158, 157, 154 (<u>C</u>-OH), 145, 133, 130 (5 C), 127 (4 C), 120, 115 (2 C), 111, 94 (3 C), 67, 66 (3 C), 37, 35, 31 (2 C), 29, 27, 24, 23, 22 (2 C). HRMS (FAB) calc. for $[M+H]^+$ 611.34871, found 611.3501. Analysis calc. for $C_{40}H_{50}O_7N_2$: C 71.62; H 7.51; N 4.18. Found: C 71.62; H 7.50; N 3.94.

6: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (d, J = 6.6 Hz, 6 H, $CH(C\underline{H}_3)_2$), 1.65 (m, 2 H, CH-$C\underline{H}_2$), 1.80 (m, 1 H, C<u>H</u>), 2.66 (s, 3 H, N=C-$C\underline{H}_3$), 3.11 (t, J = 6.7 Hz, 2 H, $C\underline{H}_2$Ar), 3.90 (t, J = 6.7 Hz, 2 H, $OC\underline{H}_2CH_2CH$), 4.14 (t, J = 6.8 Hz, 2 H, $OC\underline{H}_2CH_2Ar$), 5.23 (s, 1 H, O<u>H</u>), 5.99-6.06 (m, 3 H, arom.), 7.38 (d, J = 8.1 Hz, 2 H, arom.), 8.00 (d, J = 8.1 Hz, 2 H, arom.). HRMS (CI) calc. for $[M+H]^+$ 383.19721, found 383.1985. Analysis calc. for $C_{22}H_{26}O_4N_2$: C 69.09; H 6.85; N 7.32. Found: C 69.24; H 6.90; N 7.23.

10: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.64 (m, 3 H, THP), 1.85 (m, 2 H, THP), 2.00 (m, 1 H, THP), 2.09 (m, 2 H, $C\underline{H}_2$), 2.18 (m, 2 H, $C\underline{H}_2$), 2.78 (t, J = 7.3 Hz, 2 H, $C\underline{H}_2$), 2.80 (t, J = 7.3 Hz, 2 H, $C\underline{H}_2$), 3.42 (t, J = 6.8 Hz, 2 H, $C\underline{H}_2$), 3.60 (m, 1 H, THP), 3.92 (m, 1 H, THP), 3.95 (t, J = 6.1 Hz, 2 H, $C\underline{H}_2$), 5.38 (t, J = 3.3 Hz, 1 H, THP), 6.77-7.19 (m, 8 H, arom.). HRMS (CI) calc. for $[M+NH_4]^+$ 450.16449, found 450.1635. Analysis calc. for $C_{23}H_{29}O_3Br$: C 63.74; H 6.74. Found: C 63.79; H 6.74.

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