Pergamon

Tetrahedron Letters 39 (1998) 3589–3592

TETRAHEDRON LETTERS

Sequencing of peptoid peptidomimetics by Edman degradation

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Received 12 February 1998; accepted 6 March 1998

Abstract

The direct identification of resin-bound peptoid peptidomimetics by sequencing is described. The N-terminus of the peptoid was treated with phenyl isothiocyanate, after which the N-terminal peptoid residue was cleaved from the resin as its thiohydantoin derivative. For deduction of the peptoid sequence, the HPLC retention times of the obtained thiohydantoins were compared to those of independently prepared reference thiohydantoins.

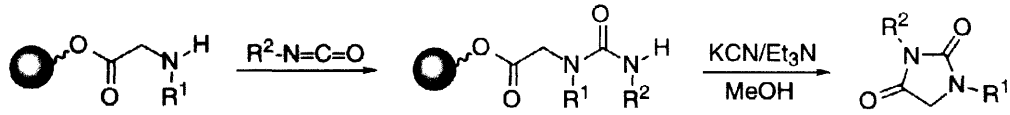
Keywords: amino acids and derivatives, peptide analogues/mimetics, isothiocyanates, thiohydantoins

The possibility to determine the sequence of a peptide or protein [1,2,3], as well as the ability to sequence nucleic acids [4,5,6], has had tremendous impacts on the development of biochemistry and molecular biology. More recently, sequence determination has become a very important method for establishing the identity of a compound of a split-mix combinatorial library when compounds are needed with a particular biological activity *e.g.* in the drug discovery process [7,8]. The peptide or nucleic acid present in a library, which is subjected to sequencing, can be either the compound possessing the required biological properties or function as a coding tag for a different compound. In the majority of cases sequencing as a tool for direct identification of a compound in combinatorial libraries is not possible and to remedy this sophisticated and elegant tagging technologies have been developed in order to encode for the identity of a compound [9]. However, direct chemical identification of a library member by sequencing or direct spectroscopic identification remains a preferred approach.

In this communication we show that in addition to the well-established methodology of sequencing **peptides** by Edman degradation, it is now possible to sequence a particularly interesting class of -oligomeric- peptidomimetics denoted as **peptoids** [10,11,12]. In general, the purpose of developing peptidomimetics is to obtain compounds which posses the disadvantages of peptides to a lesser extent, while retaining their biological activity. As such they are interesting in the drug discovery process and the possibility to identify a peptoid library member on solid phase beads by direct sequencing would be very attractive indeed.

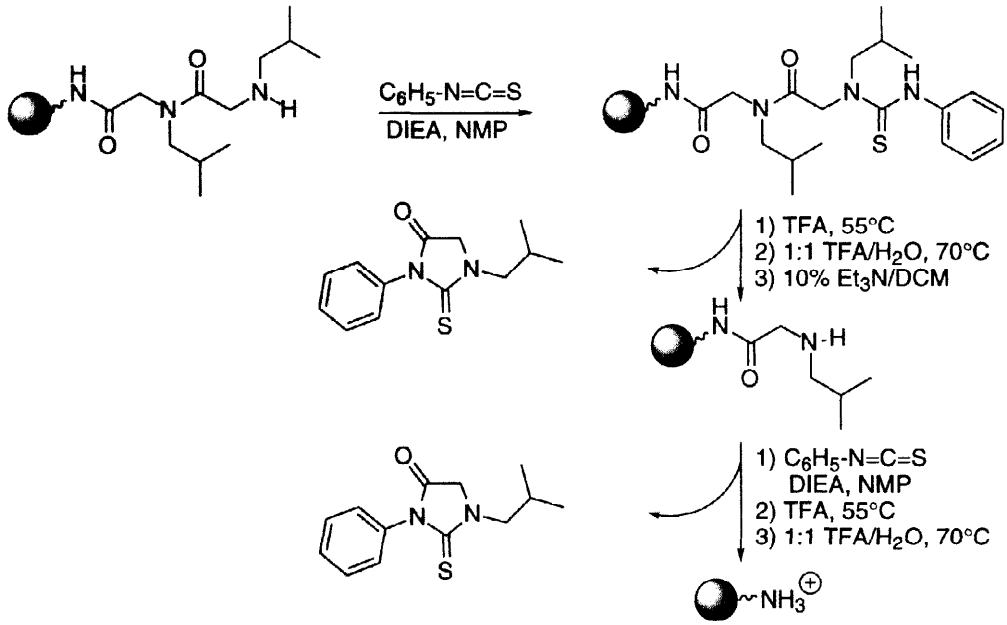
Recently we found that it is possible to prepare a diversity of (thio)hydantoins by cyclization-cleavage from the solid phase (Scheme 1) [13]. This included hydantoins which were obtained from N-alkylated glycine derivatives. Since peptoids are composed of N-alkylated glycine derivatives, we wondered if it would be possible to prepare a thiohydantoin derivative from an N-alkylated glycine residue attached to an amino-function in stead of a hydroxy function. If this would be the case we would have a tool to cleave off an N-alkylated amino acid from *e.g.* a preceding N-alkylated amino acid, and thus the ability to *sequence peptoids.*

0040-4039/98/$19.00
PII: S0040-4039(98)00556-5



Scheme 1. Synthesis of hydantoins starting from resin-bound N-alkylated glycine residues

As a pilot sequence to investigate our concept we took a NLeu-NLeu peptoid dimer immobilized on a Tentagel®-NH_2 resin (Scheme 2). The N-terminus of the peptoid was treated with a six fold excess of phenyl isothiocyanate and one equivalent of DIEA in NMP for 1.5 hr. At the end of the reaction, excess reagents were removed by filtration, and the resin was washed with NMP and DCM. After drying under vacuum, the resin was stirred in TFA at 55°C for 15 minutes. The resin was filtrated and washed with TFA to collect the thiazolinone, after which water was added to the filtrate in a one to one ratio. This solution was stirred at 70°C for 15 minutes to effect conversion of the thiazolinone into the thiohydantoin. Evaporation of the solvent gave the product thiohydantoin in 91% yield, as was confirmed by NMR analysis. Subsequently, the resin was neutralized by washing with TEA and subjected to a next round of treatment with phenyl isothiocyanate to give the expected thiohydantoin.



Scheme 2. Sequential removal of thiohydantoins from an immobilized NLeu-NLeu peptoid

Encouraged by the results of this experiment, which clearly showed that sequential removal of residues from a peptoid peptidomimetic by degradation under Edman conditions was possible, we embarked on the preparation of a large set of reference thiohydantions. These thiohydantoins were derived from the peptoid monomers which are routinely prepared for our solid phase peptoid synthesis [14]. The reference compounds were the thiohydantoin derivatives of the following peptoid monomers: Fmoc-Gly-OH, Fmoc-NAla-OH, Fmoc-NPhe-OH, Fmoc-NLeu-OH, Fmoc-NIle-OH, Fmoc-NVal-OH, Fmoc-Pro-OH, Fmoc-NMet-OH, Fmoc-NLys(Boc)-OH, Fmoc-NOrn(Boc)-OH, Fmoc-NArg(Boc)2-OH, Fmoc-hNTrp(Boc)-OH, Fmoc-hNSer(OtBu)-OH, Fmoc-NAsp(OtBu)-OH and Fmoc-NTyr(OtBu)-OH.

Coupling of the peptoid residues to Tentagel®-NH_2 and removal of the protecting groups gave the unprotected resin-bound peptoid residues. These were converted into the thiohydantoins according to the procedure described above. All thiohydantoins were obtained in reasonable to good yields (60 to 100%).

For an efficient sequential analysis based on determination of the retention time of the cleaved off thiohydantoin, it is essential that most of the thiohydantoins possess a different retention time in one or two HPLC-buffer systems. We were very fortunate to find a gradient protocol which could separate *all* thiohydantoins from each other (Figure 1)![1]

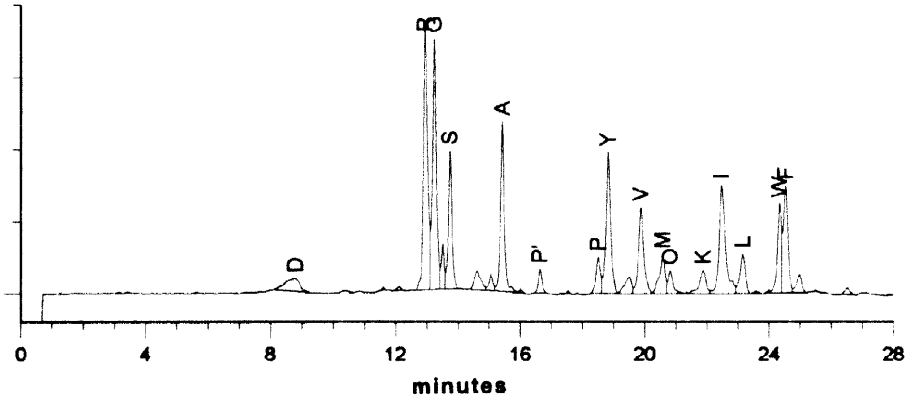


Figure 1. HPLC trace of a mixture of the reference hydantoins

The availability of the appropriate reference thiohydantoins and the possibility to separate them by HPLC set the stage for carrying out actual sequencing. For this purpose we chose the peptoid peptidomimetics of Leu-enkephalin (YGGFL) [15,16] and the C-terminal pentapeptoid corresponding to the Substance P C-terminal peptide (FFGLM) [17,18].[2] Sequencing of both peptoids went very well and their sequences could be deduced unambiguously based on the retention times of the reference thiohydantoins. (Figure 2).

It is no problem whatsoever to reduce the amount of resin used for sequencing substantially. The amount of resin could be reduced easily from 250 mg to 10 mg and 1 mg and sequencing of the peptoid of Leu-enkephalin *i.e.* nYGGnFnL proceeded without any difficulties. Therefore, we are confident, that ultimately it will be possible to determine the sequence of a peptoid present on a single bead.

In conclusion, we have developed a method for sequencing peptoid peptidomimetics by Edman degradation, which allows for the rapid identification of resin bound peptoids as was demonstrated by the successful sequencing of nYGGnFnL and nFnFGnLnM. Moreover, we have shown that the amount of resin to be used for sequencing can be reduced without any difficulty, thereby offering perspectives for sequencing on a single bead. As such this will represent a direct approach for establishing the identity of a compound from a combinatorial library, without the need of sophisticated encoding or elaborate deconvolution procedures. Finally, providing that one is able to find the appropriate HPLC gradient protocols, this approach will also be suitable for the identification of peptide-peptoid hybrids, which adds even more to the collection of useful tools available to the combinatorial chemist.

1. Thiohydantoins show a specific absorbtion at 270 nm. Using this wavelength for detection has the advantage that possible impurities will not interfere with a correct identification of the compounds.
2. The peptoid peptidomimetics of YGGFL and FFGLM *i.e.* nYGGnFnL and nFnFGnLnM, respectively, were prepared on Tentagel®-NH_2 according to references 10 and 14.

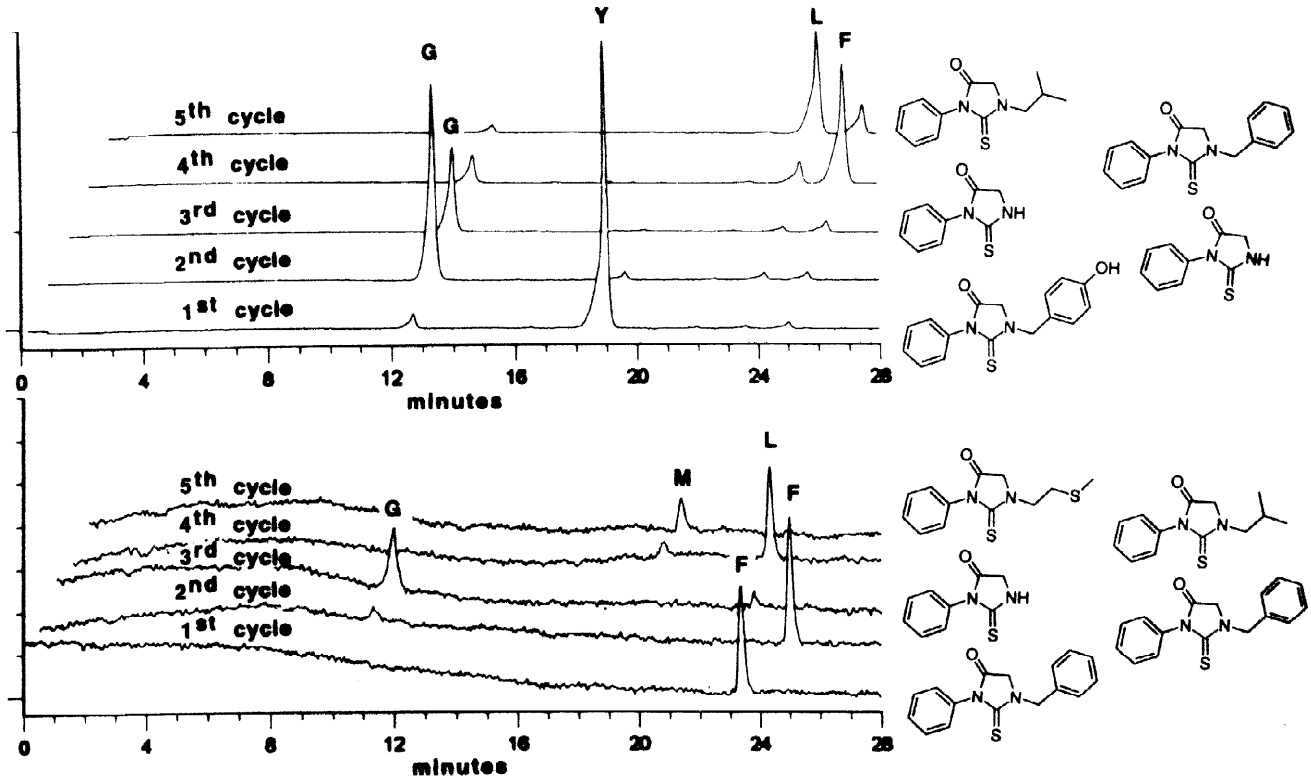


Figure 2. HPLC traces of the thiohydantoins, which are obtained after cleavage of subsequent N-terminal peptoid residues from the peptoid corresponding to Leu-enkephalin (YGGFL, top) and to the Substance P C-terminal peptide (FFGLM, bottom)

Acknowledgments

Financial support for this work was obtained from Solvay Pharmaceuticals. We thank Mr Mesut Özturk, Ms Lovina Hofmeyer and Dr John Kruijtzer for synthesizing the starting peptoid monomers.

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