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Solid-phase synthesis of benzodiazepinediones mimicking the C-terminus of the H-Ras protein

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ABSTRACT

A combined solution and solid-phase method for the synthesis of benzodiazepinediones mimicking the lipid-modified C-terminus of the H-Ras protein was developed. By means of this 14-step sequence, a collection of 42 peptidomimetics was synthesized.

1. Introduction

The Ras proteins are membrane-bond GTPases that are key regulators of important biological processes, in particular, transduction of growth-promoting signals across the plasma membrane.[1] The H- and N-Ras isoforms are S-palmitoylated and S-farnesylated at the C-terminus, which results in membrane anchoring and is a prerequisite for full biological activity. Interference with the enzymatic machinery that regulates Ras lipidation has proven to be a viable approach to the study of Ras biochemistry, -biophysics, -structural, and -cell biology.[2–4]

For the development of small molecule modulators of Ras function and processing,[3,5] we recently described the synthesis of a class of compounds that mimick the structure of the H-Ras C-terminus.[2] The design included a benzodiazepinedione as central peptidomimetic unit representing the C-terminal amino acid sequence of H-Ras, and two variable building blocks mimicking a S-palmitoylated and S-farnesylated cysteine methyl ester, respectively, and led to the discovery of compounds that interfere with the neurite outgrowth promoting activity of Ras in PC12 cells. The benzodiazepinedione core was chosen because it can be regarded as a 'privileged' structure, which has proven valuable in several different applications.[6,7]

We now report on the development of a combined solution and solid-phase approach to the synthesis of a H-Ras mimicking benzodiazepinedione collection based on the structures of the previously identified modulators of cellular Ras activity.

2. Results and discussion

The synthesis was designed to allow rapid access to benzodiazepinediones with two variable building blocks as shown in Figure 1.

Diversity is introduced at the variable sites via formation of amide and/or sulfonamide bonds in the last steps of the synthesis. According to our initial findings, the free amino group attached to the heterocyclic core is important for high biological activity.

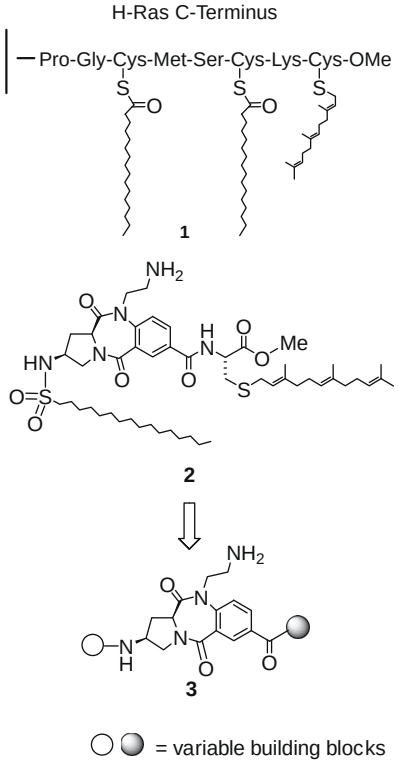

Figure 1.

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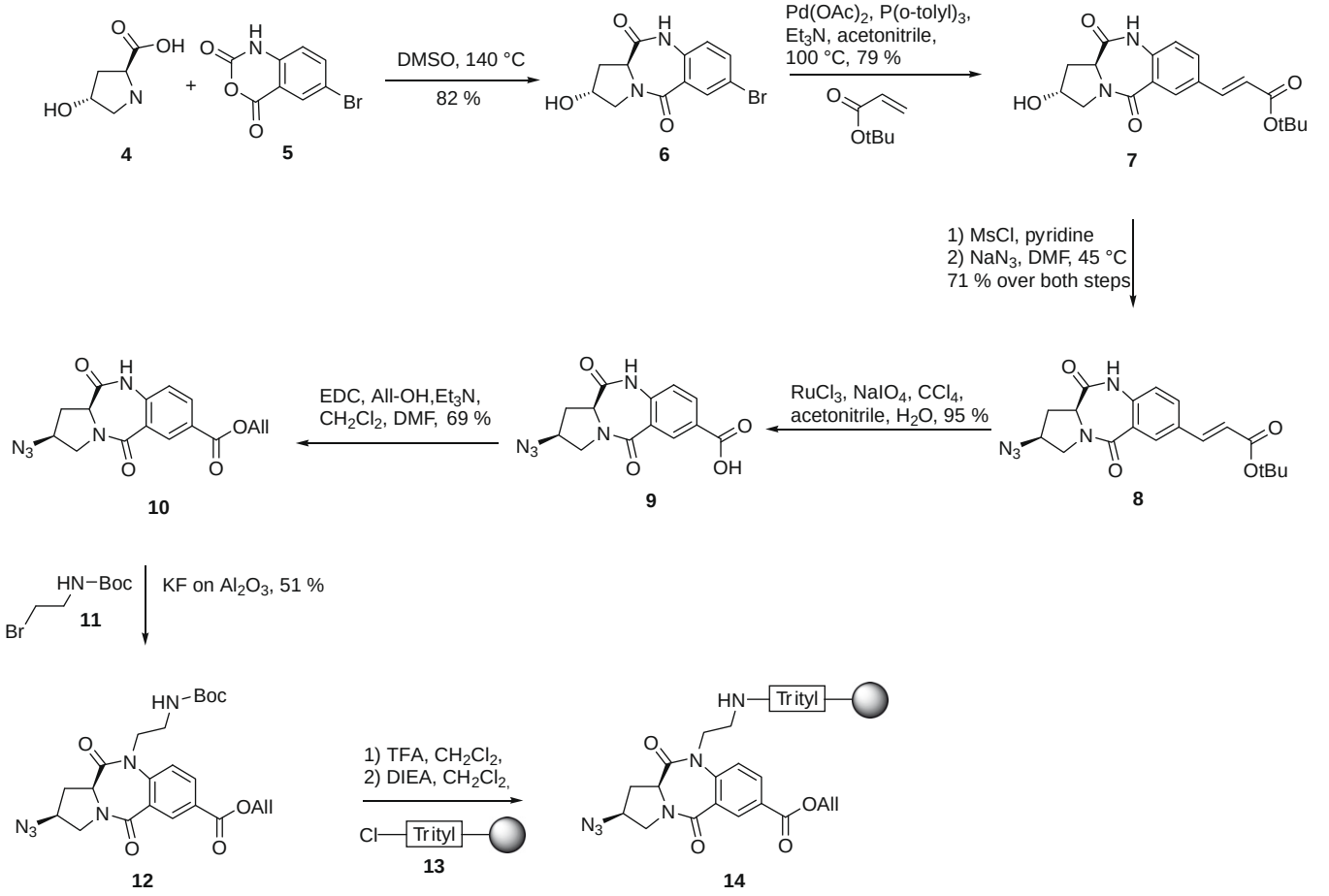


Scheme 1.

Therefore, it was preserved in the library and was chosen for attachment of the molecules to a solid support.

The synthesis of farnesylated peptides is complicated by the acid lability of the farnesyl group.[8] Thus, for the solid-phase synthesis of farnesylated compounds, such as **1**, only very mildly acidic conditions are tolerable limiting the number of possible solid-phase linkers and reaction conditions. We, therefore, decided to use the very acid labile Trityl group as linker to a solid support since attachment of lipidated peptides via side chain primary amino groups reliably gives access to farnesylated peptides.[9]

The benzodiazepinedione scaffold was prepared as shown in Scheme 1.

Treatment of bromoisatoic anhydride **5** with 4-(*R*)-hydroxy-l-proline **4** in DMSO provided benzodiazepinedione **6** in 82% yield.[10] Allylester **10** was prepared by a five-step procedure. First, a Heck reaction with *tert*-butyl acrylate afforded compound **7** in 79% yield. The hydroxy group was mesylated, and then converted into the azide **8**. The double bond was oxidatively cleaved with $RuCl_3/NaIO_4$ to give carboxylic acid **9**. Compound **9** was esterified to give **10** with EDC and allyl alcohol in the presence of DMAP.

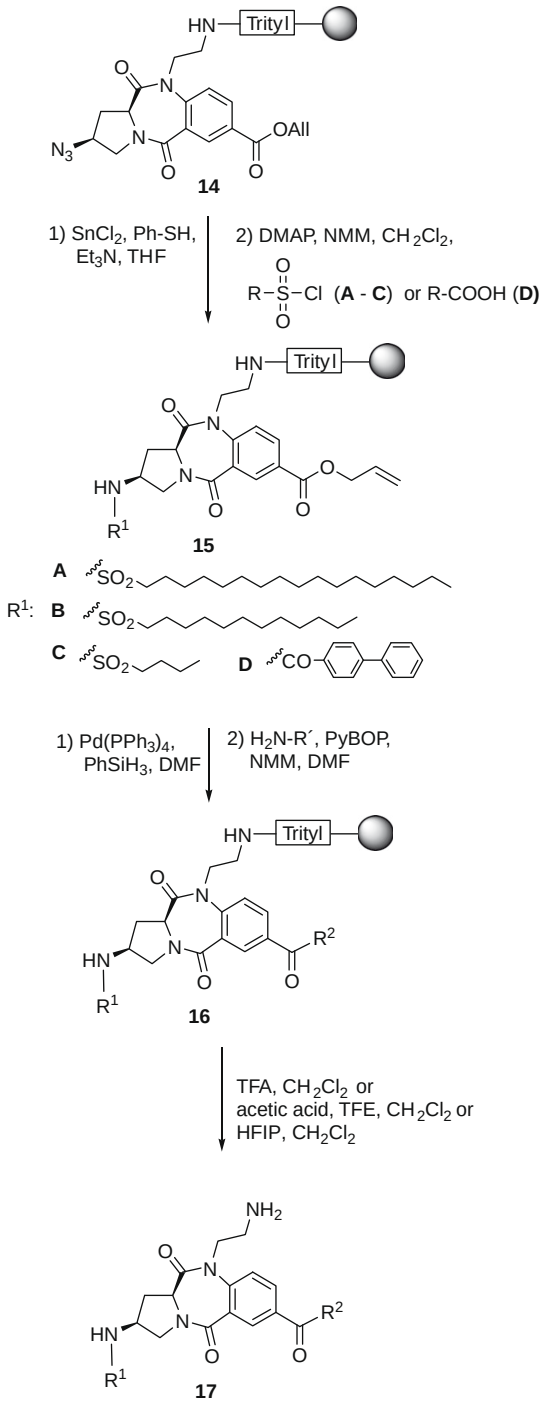
Benzodiazepinedione **10** was then alkylated under mild conditions with 2-(Boc-amino)ethyl bromide **11** in the presence of potassium fluoride on aluminium oxide to yield benzodiazepinedione **12**. The use of NaH for deprotonation of the amide was not successful. During alkylation, some epimerization was observed, which makes purification by means of preparative HPLC necessary. Compound **12** was obtained in 51% yield. This fully protected benzodiazepinedione was then immobilized on solid support. To this end, the Boc-group was cleaved with TFA, and the resulting free primary amine was reacted with a trityl chloride resin **13** to obtain polymer-bound benzodiazepinedione **14**. The chemically orthogonal combination of an amide with an allyl-protected carboxylic acid opens up a viable opportunity for the flexible selective derivatization of the benzodiazepinedione core after liberation of the functional groups.

Reduction of the azide was achieved with tin(II)chloride and thiophenol under basic conditions (Scheme 2).

Alternatively, tributyl phosphane/water could be employed. The free amine was then equipped with different building blocks. Treatment with sulfonyl chlorides together with *N*-methyl-morpholine (NMM) and DMAP according to Gennari et al.[11] yielded sulfonamides **15**. Repeated coupling proved to be necessary for full conversion. In addition, a biphenyl was introduced by coupling of 2-biphenylacetic acid with HBTU, HOBt, and DIEA (not shown in Scheme 2).

After the introduction of the first building block, the allyl ester of **15** was cleaved with $Pd(PPh_3)_4$ in the presence of phenylsilane as allyl trapping nucleophile. The resulting free carboxylic acid was activated with PyBOP and NMM and reacted with different amines as the second building blocks to give polymer-bound target molecules **16** (Scheme 2). The corresponding biphenyl derivatives were obtained accordingly.

Inspired by our initial findings,[3] lipophilic groups mimicking the palmitic acid thioester incorporated into H-Ras were introduced at the N-terminus of the compound collection. In contrast, the C-terminus was equipped with different functional groups including differently lipidated cysteines, alkyl amines, aromatic

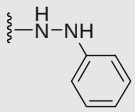
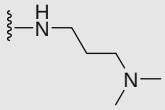
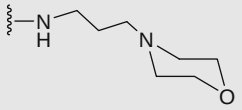


Scheme 2.

molecules such as phenylalanine methyl ester, benzyl amine, or phenylhydrazide as well as basic functionalities (Table 1).

Release of the products **17** from the resin was achieved by treatment with 2% TFA in dichloromethane (five times, 5 min each). After purification, the desired products were obtained in 16–59% yield for six steps on the polymeric support. For compounds incorporating cysteine building blocks, the use of acetic acid and triethylsilane (TES) or hexafluoro-2-propanol (HFIP) in dichloromethane gave superior results. Selected examples are shown in Table 1, for all synthesized compounds see Supplementary data.

Table 1
Selected results of the synthesis of the benzodiazepinedione collection

No.	R^1	R^2	Yield (%)
17/12	D	–Ala-OMe	63
17/19	A	–NH-*n*-C_6H_{13}	50
17/25	A	–Cys(Far)-Ome	59
17/31	C	–Cys($C_{16}H_{33}$)-Ome	43
17/35	A		16
17/38	B		35
17/42	D		60

3. Conclusion

We have shown that differently functionalized benzodiazepinediones mimicking the lipidated C-terminus of the H-Ras protein are accessible by means of a combination of solution and solid-phase methods including six steps on the solid support. A total of 42 target molecules were synthesized with overall yields of 16–59% for the last seven steps of 16–59%. The obtained lipidated peptide mimetics will be subjected to chemical–biological investigations of Ras function.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.01.016.

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