Organic LETTERS

Letter

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Synthesis of the TACO Scaffold as a New Selectively Deprotectable Conformationally Restricted Triazacyclophane Based Scaffold

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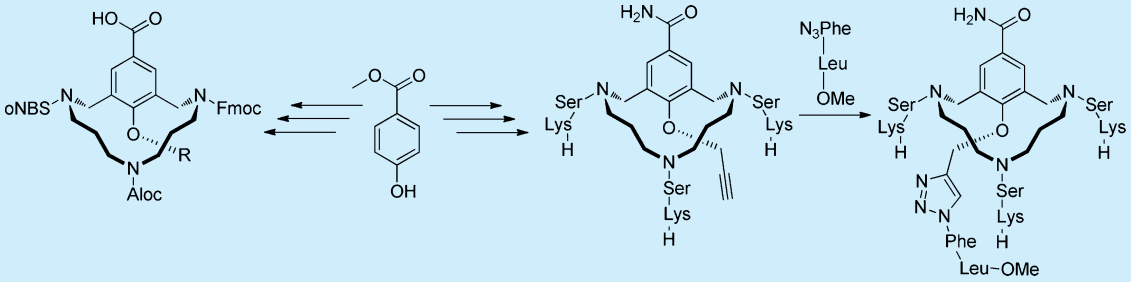
Supporting Information



ABSTRACT: The synthesis of a new triazacyclophane scaffold (TACO scaffold) containing three selectively deprotectable amines is described. The TACO scaffold is conformationally more constrained than our frequently used TAC scaffold, due to introduction of a substituent on the para position of the benzoic acid hinge, which prevents ring flipping and makes it more attractive than the TAC scaffold for preparation of artificial receptor molecules or for mimicking discontinuous epitopes toward protein mimics when more preorganization is required.

Molecular scaffolds have been described in the literature to which three or more *identical* ligands such as peptides can be attached, but only a few are suitable for the selective attachment of three *different* ligands.[1] The TAC (TriAzaCyclophane) scaffold, developed in our group, was one of the first selectively deprotectabe scaffolds.[2] This scaffold consists of a benzoic acid hinge connected to a symmetric cyclophane bridge containing three secondary amines, which carry three different selectively removable protecting groups (Figure 1). We have used the TAC scaffold for the development of synthetic receptors,[3,4] synthetic vaccines[5] involving mimicry of discontinuous epitopes,[7,8] and combinatorial libraries,[3,4,6] for example toward artificial enzymes.[6] Toward the mimicry of a discontinuous epitope of HIV-gp120, we compared a small number of chemically diverse scaffolds, which underlined the importance of the nature of scaffolding.[8] Using different approaches we are now able to introduce different ligands, especially (cyclic) peptides, onto our scaffolds and therefore we can now increasingly focus our attention on the much more difficult issue of attempting to orient the ligands with respect to each other in space. In order to try to achieve this we have so far (1) varied the rigidity of the scaffold[8] and (2) attempted to influence the conformational space of the cyclophane ring and therefore the orientation of the ligands, by changing its ring size. Here we describe the "TACO" scaffold, a new O-alkylated TAC-scaffold, in which the O-alkyl substituent may function as an important orientation director (Figure 1). The TAC scaffold consists of a benzoic acid hinge connected to a symmetric bridge containing three amine functional groups with semi-orthogonal protecting groups. Naturally, despite the presence of the benzene ring as part of these large (14-membered) cyclophane macrocycles, they are very flexible. As a consequence, ring flipping occurs easily in these macrocycles[9] leading to different conformers. In order to reduce ring flipping, the triazacyclophane ring was reduced by one CH_2 moiety, which led to the A(symmetric)TAC scaffold.[10] The ATAC scaffold was less flexible, but not surprisingly, ring flipping still occurred. We envisioned that, by introduction of a substituent on the para position of the aromatic ring, thus affording a TAC-O-alkylated molecular scaffold (Figure 1), ring flipping might

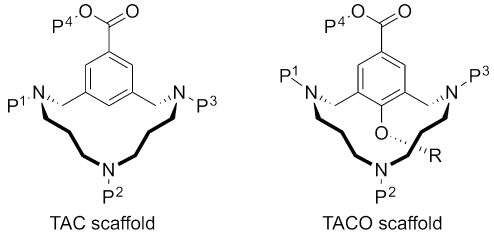
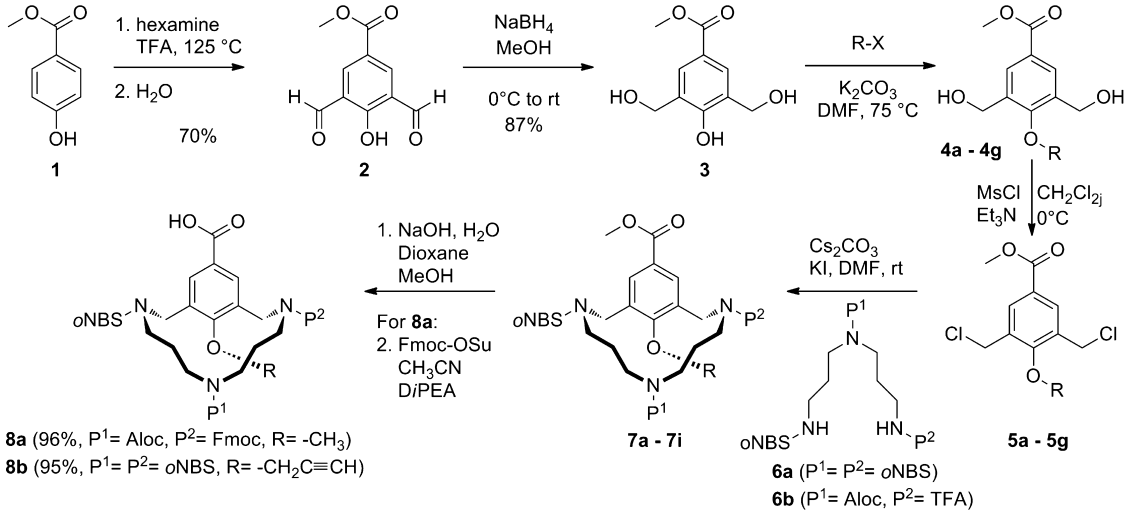


Figure 1. TAC scaffold vs TACO scaffold.

Received: April 29, 2014
Published: May 23, 2014

   dx.doi.org/10.1021/ol5012218 | *Org. Lett.* 2014, 16, 3106–3109

Scheme 1. Synthesis of Different TACO Scaffolds



be further reduced. Both simple physical molecular models and preliminary molecular modeling experiments (See Supporting Information) show that an O-alkyl group pushes P^1 and P^3 upward, whereas P^2 will stay down (Figure 1).

In principle substituents such as alkyl, amine, ether, and ester can be introduced. However, an ether linkage was preferred since it is very stable, neutral, and easily accessible. Instead of introducing the alkoxy substituent at the para (4) position of 3,5-dimethylbenzoic acid, which is the starting material in the TAC scaffold synthesis,[10] it was decided that the methyl groups would be introduced at both meta (3 and 5) positions of methyl 4-hydroxybenzoate (**1**, Scheme 1). This was synthetically attractive, and the use of methyl 4-hydroxybenzoate as a starting material was also substantially cheaper than using 3,5-dimethylbenzoic acid.

Thus, methyl-4-hydroxybenzoate (**1**) was subjected to a modified Duff reaction[11] by treatment with hexamine in refluxing TFA as a solvent, leading to bis-formylated benzoate **2** in 70% yield (Scheme 1). For the possibility of coupling an alkyl group to the phenolic−OH at the last stage of the synthesis, temporary protection was attempted using silyl protecting groups (TBDMS and TMSE). Unfortunately, all attempts were unsuccessful, pointing to the low nucleophilicity of the phenolic−OH, likely due to delocalization of the negative charge of the phenolate on both aldehyde functionalities in **2**. To increase its nucleophilicity, it was decided that both aldehydes of **2** would be reduced using $NaBH_4$ in methanol, affording trihydroxy benzoate **3** in high yield (87%). Selective protection of a phenolic−OH in the presence of benzylic alcohols is impossible,[12,13] therefore alkylation of the phenolic−OH was decided for this stage of the synthesis, since the resulting ether moiety will be inert to subsequent synthetic transformations. Standard phenol alkylation conditions using an alkyl halide in acetone or DMF with K_2CO_3 as a base[14,15] were optimized leading to treatment with a slight excess of the alkyl bromide (1.2 equiv) in DMF at 75 °C (Table 1). Good to high yields (66% to 100%) were obtained with several alkylating agents. Not surprisingly, the methyl ether (**4a**) was prepared in high yield too. Alkylation with functionalized alkyl bromides Boc-2-bromoethylamine and propargyl bromide also proceeded smoothly leading to ethers **4f** and **4g**, which now contained an additional handle for convenient functionalization (CuAAC, thiol−ene, conjugation, etc.) after completion of a TACO-molecular construct.

Table 1. Yields of Alkylations of 3 with Different Alkyl Halides (R−X), Affording 4a−4g (1 equiv of 3, 1.2 equiv of alkyl halide, and K_2CO_3, DMF, 75 °C)

entry	R-X	time (h)	product	yield (%)
1[a]	MeI	5	**4a**	88
2[b]	Br	2	**4b**	87
3	Br	2	**4c**	91
4	Br, NO_2	0.75	**4d**	quant
5	Br, *t*Bu, *t*Bu	1	**4e**	75
6	Br	3	**4f**	66
7	Br, NHBoc	1	**4g**	95

[a]Temperature: 40 °C. [b]Temperature: 50 °C.

In attempts to equip the benzyl alcohols with mesylate leaving groups, bis-benzylic alcohols **4a−4g** were reacted with methanesulfonyl chloride and triethylamine as a base. Not unexpectedly, no bis-mesylates were found, but instead bis-benzylic chlorides **5a−5g** were isolated[16] which were sufficiently pure for direct use in the macrocyclization reaction leading to **7a−7i** (Scheme 1). For evaluation of the scope of this reaction, the more easily accessible triamine containing three *o*NBS-groups (**6a**, $P^1 = P^2 =$ *o*NBS) was used.[3] The macrocyclization reaction was performed using 1 equiv of both the bis-benzylic chloride and triamine **6a** in DMF, in the presence of potassium iodide and cesium carbonate as a base. The macrocyclization reaction using the bis-benzylic chlorides **5a−5g** proceeded equally well as when using a bis-benzylic bromide,[10] and TACO scaffolds **7a−7g**, including those containing groups suitable for further functionalization (**7f** and **7g**), were obtained in moderate yields (42−67%, over two steps) (Table 2), after column chromatography. Even TACO

dx.doi.org/10.1021/ol5012218 | Org. Lett. 2014, 16, 3106−3109

scaffold **7e** with the sterically demanding 3,5-di-*tert*-butylbenzyl group was afforded in a moderate yield of 51%.

Table 2. Yields of TACO Scaffolds 7a–7i after the Macrocyclization Reaction with Protected Triamines 6a (entries 1–7) and 6b (entries 8–9) (1.0 equiv of 5a–5g and 6a or 6b, 4.0 equiv of Cs_2CO_3, 0.1 equiv of KI, DMF, rt)

entry	R	P1	P2	product	yield (%)
1	-Me	*o*NBS	*o*NBS	**7a**	59
2		*o*NBS	*o*NBS	**7b**	67
3		*o*NBS	*o*NBS	**7c**	51
4	NO_2	*o*NBS	*o*NBS	**7d**	67
5	*t*Bu, *t*Bu	*o*NBS	*o*NBS	**7e**	51
6	NHBoc	*o*NBS	*o*NBS	**7f**	42
7		*o*NBS	*o*NBS	**7g**	51
8[a]		Aloc	TFA	**7h**	41
9	-Me	Aloc	TFA	**7i**	49

[a] CH_3CN, reflux, 1 h.

After these successful macrocyclizations, triamine **6b**,[10] containing now three different protecting groups (P^1 = Aloc, P^2 = TFA), was used in this alkylation reaction with bis-benzylic chlorides **5a** and **5f**, and TACO scaffolds **7h** and **7i** were indeed obtained in reasonable yields (41% and 49%, respectively). Finally, saponification of the methyl ester in scaffolds **7g** and **7i**, and simultaneous cleavage of the TFA-group (in **7i**), followed by introduction of an Fmoc-group (for preparing **8a**) afforded TACO scaffolds **8a** and **8b** in high yields (96% and 95%, respectively), after column chromatography.

To investigate whether an additional substituent indeed can be introduced on the alkyne group of a TACO scaffold already containing three peptides, TACO-scaffold **10** was synthesized. Thus, TACO-scaffold **8b** was coupled to Tentagel resin containing a Rink amide linker, using BOP as a coupling reagent and D*i*PEA as a base, affording resin **9** (Scheme 2). Removal of the *o*NBS-groups by 2-mercaptoethanol and DBU in DMF was followed by subsequent coupling of Fmoc-Ser(*t*Bu)-OH and Fmoc-Lys(Boc)-OH using BOP and D*i*PEA. Removal of the Fmoc-groups by piperidine and then acetylation afforded TACO-tris-dipeptide **10**. Cu(I)-assisted azide–alkyne cycloaddition (CuAAC)[17,18] was carried out after treatment of resin **10** with TFA/TIS/H_2O to afford **11**, which was purified by preparative HPLC. Reaction with azidoPhe-Leu-OMe (**12**), using $CuSO_4$ and sodium ascorbate in a DMF/water mixture under microwave irradiation conditions, afforded TACO scaffold **13** now containing four peptides in a good yield of 66%, after preparative HPLC.

To determine whether an additional substituent at the para position of the benzoic acid hinge is capable of inhibition of the ring flipping process, TACO scaffold **7a** was compared with a TAC scaffold containing three *o*NBS-groups (Figure 2). Ring flipping can be easily monitored using variable temperature ^1H NMR experiments (Figure 3).[9,10]

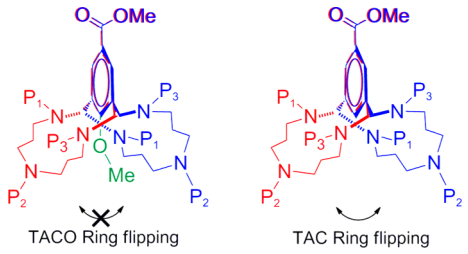
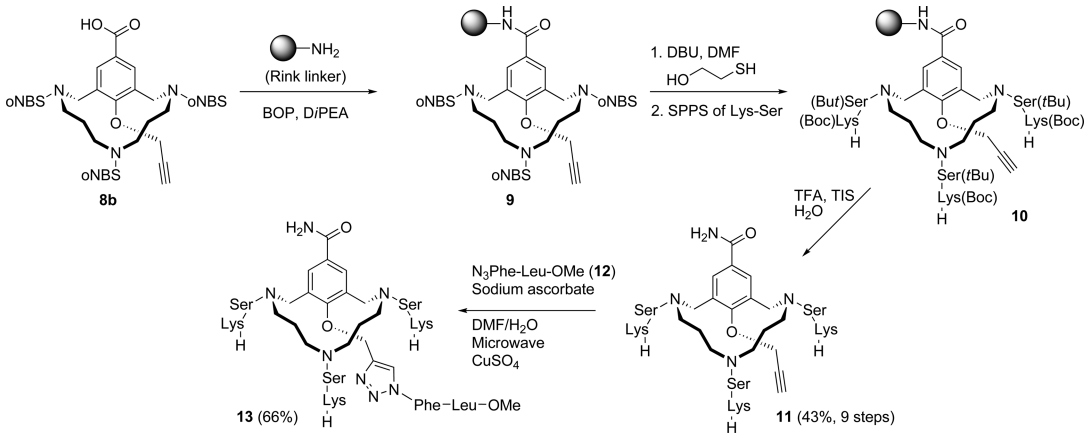


Figure 2. Superimposed ring flipping isomers of the TACO and TAC scaffolds (P_{1-3} = *o*NBS).[3]

At room temperature, the benzylic CH_2 groups in the scaffolds were visible as a singlet for the TAC scaffold and an AB system for the TACO scaffold. This at least indicated that the TAC scaffold can ring flip easily at rt, but that the TACO scaffold is conformationally more restricted because the benzylic protons are magnetically nonequivalent. The singlet of the TAC scaffold starts to broaden significantly after cooling to −80 °C, indicating slower ring flipping. The TACO scaffold was measured at a high temperature (110 °C), in order to stimulate ring flipping. Surprisingly, there was hardly any

Scheme 2. Attachment of Three Dipeptides to the Triazacyclophane Ring and an Additional Peptide Connected to the Alkyne Moiety Using CuAAC Chemistry



dx.doi.org/10.1021/ol5012218 | *Org. Lett.* 2014, 16, 3106–3109

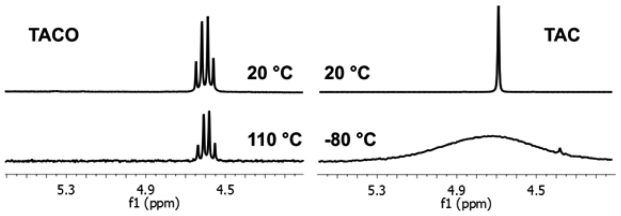


Figure 3. Variable temperature ^{1}H NMR signals for the diastereotopic benzylic CH_2 protons from TACO scaffold **7a** and the TAC scaffold (Figure 2, P_{1-3} = *o*NBS).

change in the ^{1}H NMR spectrum. The coupling constants for the AB systems of the benzylic CH_2 groups were identical at both temperatures, showing that even at high temperatures likely no ring flipping occurred in a TACO scaffold even containing a substituent as small as a methoxy group at the 4-position of the benzoate hinge (**7a**). These results indicate that the TACO scaffold may be promising for use as a more preorganized scaffold for the construction of protein mimics.

In conclusion, we have described an efficient and high yielding method for the multigram scale synthesis of a new (selectively deprotectable) and conformationally more restricted TACO scaffold. Although this scaffold is very similar to the TAC scaffold frequently used by us, the ether substituent at the 4-position of the benzene ring has a great influence on the flexibility of the cyclophane ring. VT NMR measurements showed that indeed the TACO scaffold is more preorganized than the TAC scaffold. In addition, a suitably functionalized ether substituent at this position provided an anchoring position for another substituent, so that four different ones can be introduced lining the cyclophane ring, while there is still the possibility of a fifth substituent to be incorporated onto the carboxyl moiety of the benzoate. We believe we have provided a very valuable addition to our repertoire of scaffolds, which can be applied to the synthesis of highly functionalized protein mimics or other densely functionalized (bio)molecular constructs. The present investigation includes their application in the assembly of synthetic vaccines and other challenging biomimics.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and characterization of the products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We would like to thank Dr. Johann Jastrzebski and Dr. Johan Kemmink, for the help with the variable temperature NMR measurements, and Dr. Ir. D. T. S. Rijkers and Dr. Remco Merkx, for the kind gift of azidopeptide **12**.

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