DOI: 10.1002/cmdc.200800321

Assessing the Bioisosterism of the Trifluoromethyl Group with a Protease Probe

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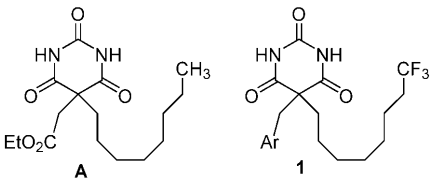
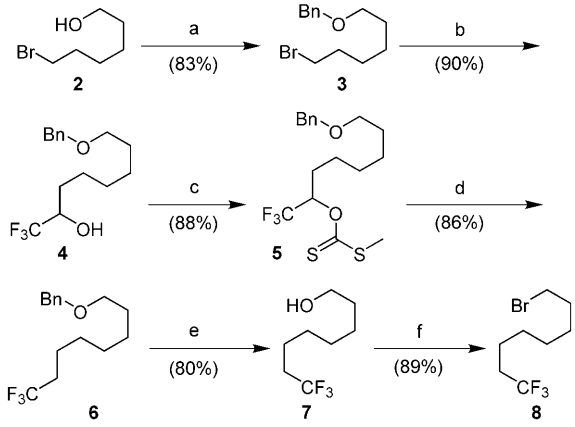
The bioisosterism of the trifluoromethyl group, namely its capacity to act as a replacement for groups with similar size or shape without substantially altering key biological properties such as binding affinity, remains a controversial issue. Until recently the most accepted idea was that CF_3 and isopropyl groups are interchangeable, whereas CF_3 was thought to be considerably bulkier than CH_3.[1] However, a recent theory supported by careful analysis of van der Waals volumes and shapes of the CF_3 group in comparison with various alkyl groups has suggested that CF_3 is closer to the ethyl group in terms of steric effect, whereas the isopropyl group is larger.[2] Considering the importance of the CF_3 group in medicinal chemistry and drug discovery,[3] we decided to investigate the issue of CF_3 bioisosterism further, and to clarify it using an empirical "lock and key" approach.[4] In fact, according to Müller et al.,[2] replacement of alkyl residues by similarly sized fluoroalkyl groups in tight lipophilic pockets neither increases nor decreases binding affinity substantially. Therefore, we decided to exploit a suitable protease pocket as a steric probe to determine how effectively a CF_3 group can be accommodated in comparison with methyl, ethyl, and isopropyl groups by measuring the inhibitory potency of the corresponding molecules. The choice of the protease was critical, because the above-listed groups should be accommodated in a tight and deep hydrophobic pocket that has: 1) high affinity for CF_3 and for the selected alkyl groups, 2) stringent steric features that can discriminate between steric size and shape, and 3) the possibility to place such groups in a remote position to minimize the risk of conformational changes in the ligands, or interference by other functions of the ligands or of the protease receptor. We identified the active site of matrix metalloprotease-9 (MMP-9; gelatinase B) as the ideal probe. In fact, MMP-9 has a tunnel-like and relatively shallow hydrophobic S1′ cavity,[5] which is "shorter" than that of MMP-2 (gelatinase A, which is closely related from a structural standpoint). Furthermore, the bottom of the S1′ cavity of MMP-9 is partially blocked by the Arg 424 side chain, thus representing a potentially very selective steric probe for an MMP-9 inhibitor bearing a P1′ appendage with a CF_3 group at the ω-position.[6]

The other challenging issue was the identification of suitable inhibitors. Barbiturates have been shown to be potent and selective inhibitors of several MMPs, including MMP-9.[7] For example, compound **A** was described as a rather potent inhibitor of MMP-9 (IC_{50} = 20 nM).[8] Because the synthesis of fluorinated



analogues of **A** in our hands proved to be viable but unexpectedly complex and low yielding, we decided to investigate a less functionalized but structurally related class of 5-benzyl-5-(8,8,8-trifluorooctyl) barbiturates **1a**–**e**.[9] Barbiturates bearing 5-aryl-5-alkyl substituents have been described previously, and some show nanomolar potency toward MMP-2 and MMP-9 and selectivity versus other MMPs such as MMP-3 (stromelysin 1).[7b,c] However, the 5-benzyl-5-alkyl counterparts, to our knowledge, have not yet been reported as MMP inhibitors.

To synthesize the target barbiturates **1** we identified 1-bromo-8,8,8-trifluorooctane **8** (Scheme 1) as the key building block. This molecule is known, and was previously obtained by fluorination of 8-bromooctanoic acid with SF_4.[10] Unfortunately, the use of such an aggressive fluorinating agent requires specific experimental equipment and presents considerable safety hazards that are difficult to address in a standard academic laboratory. Alternatively, **8** was obtained by a lengthy proce-



Scheme 1. Synthesis of the key fluorinated intermediate **8**. *Reagents and conditions*: a) BnBr, NaH; b) 1. Mg, 2. CF_3CO_2Et; c) NaH, CS_2, CH_3I, THF; d) H_3PO_2, TEA, AIBN, dioxane, reflux; e) $H_2/Pd(OH)_2$, EtOAc; f) PPh_3, CBr_4, CH_2Cl_2.

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.200800321.

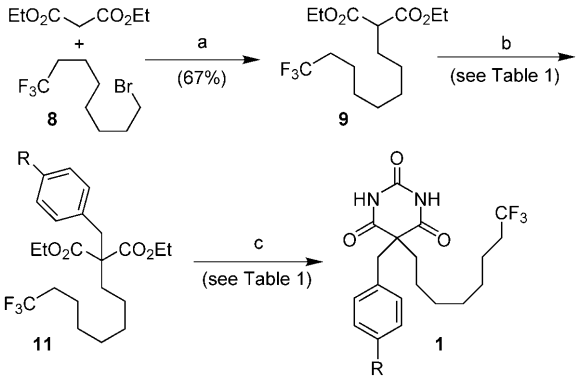
dure starting from fluorination of 1,1,1,3-tetrachloropropane by SbF_3.[11] A clear alternative would involve a cross-metathesis reaction between a CF_3-bearing olefin and a brominated olefin, but this would require the use of gaseous or highly volatile materials, particularly the trifluorinated olefin. We therefore sought to develop alternative routes to **8**, based on user-friendly protocols as well as the use of a cheap and commercially available source of fluorine, such as trifluoroacetic esters.

After considerable experimentation, several different synthetic routes to **8** with similar efficiency were developed. In one of them (Scheme 1), commercially available 6-bromohexan-1-ol **2** was O-benzylated to **3** and converted into the corresponding Grignard reagent, which was reacted according to a rather old but very efficient methodology with 0.25 equivalents of ethyl trifluoroacetate.[12] The Grignard reagent acts first as a nucleophile and than as a reducing agent, converting the intermediate CF_3-ketone into the CF_3-carbinol **4**. Barton–McCombie radical deoxygenation of the methyl xanthate **5**[13] afforded the benzyl ether **6**, which, after hydrogenolysis to the primary alcohol **7**, was converted into the target **8**.

With gram-scale quantities of the key fluorinated building block **8** in hand, we next addressed the synthesis of the barbiturates **1** (Scheme 2 and Table 1). The sodium derivative of diethyl malonate was reacted with **8** to provide **9**, which was converted into the 2,2-disubstituted malonates **11a–e** by reaction with benzyl bromides **10a–e**. Reaction with urea in the presence of *t*BuOK as base afforded the target barbiturates **1a–e**. Rewardingly, compounds **1a** (R = H) and **1b** (R = OCH_3) showed strong inhibitory potency toward MMP-9 (Table 2), and in the case of **1a** toward MMP-2 as well. Good selectivity against MMP-1 and MMP-3 was also observed, analogously to other barbiturates. Lower MMP-9 inhibitory potency was observed with **1c** (R = CH_3), **1d** (R = CF_3), and **1e** (R = Br), in decreasing order.[14] Barbiturates **1a** and **1b** were therefore identified as suitable nanomolar ligands for the next step, namely the comparison of inhibitory potency toward MMP-9 of analogues with CH_3, C_2H_5, and $CH(CH_3)_2$ groups instead of CF_3.

Following a synthetic strategy similar to that used to prepare **1a** and **1b**, the corresponding methyl, ethyl, and isopropyl barbiturates **12a–c** (R = H) and **13a–c** (R = OMe) (Table 3) were also prepared (see Supporting Information). These molecules differ from **1a** and **1b** only in the terminal R^1 alkyl group in the remote ω-position of the P1′ substituent; therefore, we anticipated that any difference in inhibitory activity must be ascribed to the different accommodation of the R^1 group at the bottom of the tight S1′ pocket of MMP-9.

The results of the inhibition tests (Table 3) unambiguously show that the isopropyl derivatives **12c** and **13c** have considerably lower activity (20–200-fold) than the CF_3-bearing ana-



Scheme 2. Synthesis of the barbiturates **1**. *Reagents and conditions*: a) NaH, 0 °C, DMF; b) 1. NaH, DMF, 2. $ArCH_2Br$ (**10**); c) urea, *t*BuOK, dry DMSO.

Table 1. Synthesis of 5-benzylbarbiturates **1**.

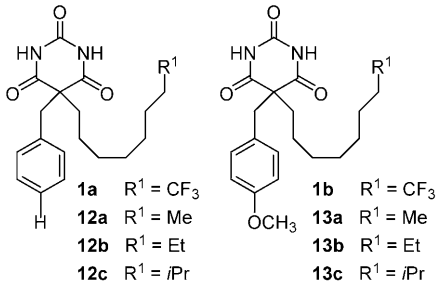
Product	R	Yields of **11** [%]	Yields of **1** [%]
1a	H	75	55
1b	OCH_3	62	37
1c	CH_3	90	93
1d	CF_3	73	67
1e	Br	80	33

Table 2. Inhibition of various MMPs.

Substrate	IC_{50} [nM][a] MMP-1	MMP-2	MMP-3	MMP-9
1a	$>10^5$	230	$>10^5$	71
1b	2.1×10^3	55	5.3×10^4	22
1c	1.8×10^4	1.9×10^3	$>10^5$	240
1d	$>10^5$	1.9×10^3	$>10^5$	1.8×10^3
1e	1.8×10^3	250	$>10^5$	530

[a] IC_{50} values represent an average of at least three titrations; assays were performed in parallel simultaneously, and standard deviations were typically within 35% of the IC_{50} values.

Table 3. Inhibition of various MMPs.



Substrate	IC_{50} [nM][a] MMP-1	MMP-2	MMP-9
1a	$>10^5$	340	87
12a	2.4×10^4	610	1
12b	$>10^5$	860	2
12c	$>10^5$	10^3	1.8×10^3
1b	2.1×10^3	55	22
13a	7.5×10^4	13	10
13b	$>10^5$	580	27
13c	$>10^5$	2.3×10^4	4.5×10^3

[a] IC_{50} values represent an average of at least three titrations, and standard deviations were typically within 35% of the IC_{50} values. Assays were carried out in parallel simultaneously, except for **1b** (see Table 2), with which a different commercial batch of MMPs from those used for the data listed in Table 2 were used.

logues **1a** and **1b**. Therefore, the isopropyl group does not fit well the S1′ pocket of MMP-9; as a bioisostere it is "larger" than the CF_3 group. In contrast, both the methyl barbiturates **12a** and **13a** were more potent than **1a** and **1b**, suggesting a better fit of the methyl group, which is definitely less sterically demanding than CF_3 in the S1′ pocket. Finally, the ethyl derivatives **12b** and **13b** were in one case more potent (~40-fold) and in the other essentially equipotent to the parent CF_3 compounds **1a** and **1b**, respectively. Notably, this trend is qualitatively confirmed by the IC_{50} values against MMP-2 as well, the S1′ pocket of which, however, is more open at the bottom, and therefore less discriminating than that of MMP-9, due to the presence of Thr424, which has a shorter side chain than Arg424 of MMP-9. The contribution of stabilizing dipolar interactions involving the hydrophobic CF_3 group and some residue of the S1′ pocket might also contribute to the observed IC_{50} values.

In conclusion, making use of a "lock and key" strategy that exploits a CF_3-bearing ligand and a tight protease pocket receptor as the steric probe, we collected IC_{50} data that support the recent hypothesis[2] on the substantial bioisosterism between the CF_3 group and the ethyl group, whereas the isopropyl group, which was previously thought to be bioisosterically equivalent to the CF_3 group,[1] appears to be "larger".

Acknowledgements

We are grateful to Prof. Wolfram Bode (Max Planck Institute, Martinsried, Germany) for useful discussions and for the crystallographic studies. We thank the European Commission (IHP Network grant "FLUOR MMPI" HPRN-CT-2002-00181, MIUR (Cofin 2004, Project "Polipeptidi Bioattivi e Nanostrutturati"), Politecnico di Milano, and C.N.R. for financial support.

Keywords: barbiturates · bioisosterism · isopropyl group · matrix metalloproteases · trifluoromethyl group

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[14] An interpretation of this strong effect of the *para* substituent on the 5-benzyl residue of the barbiturates, which should occupy the S2′ subsite of the enzymes, is difficult in the absence of structural data on the binding of these ligands to MMPs. Attempts to obtain the crystallographic structure of CF_3-barbiturates such as **1** in complex with a truncated catalytic domain of MMP-9 have been so far unsuccessful. Only a few crystallographic structures of MMP-9 complexes have been reported owing to the instability of full-length MMP-9. See also reference [6a].

Received: October 1, 2008
Revised: October 14, 2008
Published online on December 9, 2008

