ORGANIC LETTERS

2004
Vol. 6, No. 3
397−400

Synthesis of Ether Oligomers

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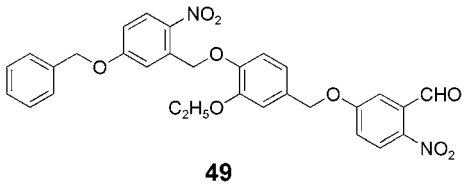
Received November 25, 2003

ABSTRACT



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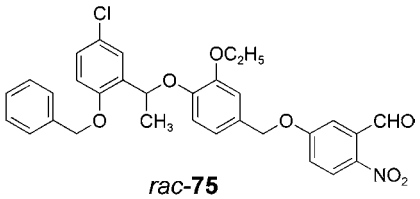
IC_{50} = 1 μM for trypsin, 3 μM for chymotrypsin, 3 μM for subtilisin



rac-**75**

IC_{50} = 3 μM for trypsin, 5 μM for chymotrypsin, 5 μM for subtilisin

Hydroxyaromatic aldehydes and ketones were used as building blocks to prepare ether oligomers. An iterative two-step protocol involving Mitsunobu coupling and carbonyl reduction provided a protecting-group-free route with high yields. Activity screening of an 84-member library against proteases led to the discovery of micromolar inhibitors for trypsin, chymotrypsin, and subtilisin.

The discovery of new bioactive organic molecules requires the exploration of molecular diversity from natural sources or by synthesis.[1] Nature very often uses peptides to bind selectively to various targets. Selectivity and potency results from the diversity created by the linear combinations of as few as 20 amino acid building blocks. The strategy has been used in numerous peptide-like oligomers containing amide-type linkages[2] but has not been exploited for assembling other types of building blocks based on different chemistries. We recently developed a new family of oligomers based on the iterative assembly of hydroxyaromatic aldehyde building blocks by oxime bond formation.[3] The potential of oxime oligomers as drug-like molecules was demonstrated by the discovery of several micromolar protease inhibitors. Herein we report a protecting group-free synthesis of ether oligomers starting from the same class of building blocks. The sequence not only gives higher yields but also is compatible with a much broader variety of building blocks. Ether oligomers display promising inhibition properties against proteases.

A key aspect of our oxime oligomer synthesis was the availability of hydroxyaromatic aldehydes with various substitution patterns, which enabled the rapid assembly of a library of oxime oligomers from commercially available building blocks and the discovery of micromolar protease inhibitors. While hydroxyaromatic aldehydes seemed well-suited to provide bioactive compounds, we encountered synthetic difficulties in expanding the scope of the oxyamination sequence. We therefore decided to redesign our oligomer synthesis by turning to a different chemistry for linkages. A new iterative coupling strategy was considered involving ether bond formation between the benzylic alcohol, available by reduction of the aldehyde group, and the phenolic hydroxyl group of the next hydroxyaromatic carbonyl compound (Figure 1). Ether bond formation between a phenol and a benzylic alcohol has been used extensively in the context of dendrimer synthesis.[4]

The Mitsunobu reaction was selected for ether bond formation since it allows activation for primary and secondary aliphatic alcohols for nucleophilic substitution with a

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(2) For selected reviews, see: (a) Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244. (b) Gante, J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1699. (c) Soth, M. J.; Nowick, J. S. *Curr. Opin. Chem. Biol.* **1997**, *1*, 120. (d) Ripka, A. S.; Rich, D. H. *Curr. Opin. Chem. Biol.* **1998**, *2*, 441.

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(4) For review, see: Fréchet, J. M. J.; Grayson S. M. *Chem. Rev.* **2001**, *101*, 3819.

10.1021/ol036300d CCC: $27.50
Published on Web 01/08/2004

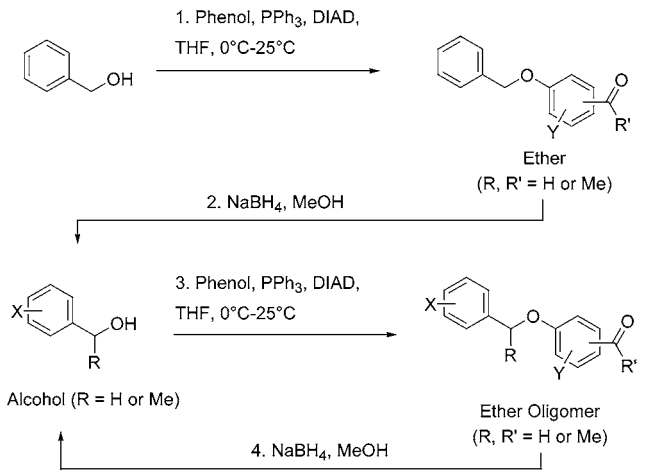

Figure 1. Iterative coupling strategy for the preparation of ether oligomers (X, Y = substituents).

broad variety of nucleophiles, including phenols.[5] The chain would start with Mitsunobu coupling between benzyl alcohol and a hydroxyaromatic aldehyde or ketone as a nucleophile, whereby the carbonyl group could be expected not to interfere with ether bond formation. After coupling, this carbonyl group could be reduced to a benzylic alcohol function for the next ether bond formation cycle.

The planned protecting group-free iterative ether bond formation sequence was implemented to form a variety of ether oligomers, including dimers, trimers, tetramers, and pentamers. Mitsunobu coupling was carried out at 0 °C in dry THF with diisopropyl azo-dicarboxylate (DIAD) and triphenyl phosphine (Ph_3P) and provided the desired ethers in good isolated yields (48−93%)[6] after purification by column chromatography. Reduction with $NaBH_4$ in methanol provided the corresponding alcohol quantitatively, which was then available for the next ether bond formation cycle. The sequence was successful for almost all building blocks tested, including ortho- and bis-ortho-substituted phenols. Acetophenones, which are available in a variety of substitution patterns, were also suitable for coupling and produced chiral compounds after reduction. Although no coupling was observed with salicylaldehyde, satisfactory yields were obtained with the corresponding 2-hydroxyacetophenone.

(5) For review, see: Mitsunobu, O. *Synthesis* **1981**, 1.

(6) Following is a typical procedure: A solution of benzyl alcohol (0.200 g, 1.85 mmol), 4-hydroxybenzaldehyde (0.226 g, 1.85 mmol), and PPh_3 (0.582 g, 2.22 mmol) was stirred in dry THF (20 mL) at 0 °C under a nitrogen atmosphere. To this mixture was added dropwise DIAD (0.44 mL, 2.22 mmol) over a period of 5 min, and the reaction was monitored by TLC. After complete disappearance of starting material (∼1 h), the solvent was evaporated under reduced pressure and the resulting oil purified by flash chromatography (hexane/AcOEt, 8/2). Oligomer **1** (0.297 g, 76%) was finally obtained as a white powder after precipitation from CH_2Cl_2/petroleum ether. Compound **1** (0.211 g, 0.99 mmol) was then dissolved in methanol (10 mL), and $NaBH_4$ (0.019 g, 0.50 mmol) was added. After stirring at room temperature for 1 h, the solution was concentrated under reduced pressure and the residue taken up with Et_2O (50 mL). The organic layer was washed (successively with water, 1 M HCl, and saturated $NaHCO_3$ solutions), dried over sodium sulfate, and evaporated. Reduced compound **2** was obtained quantitatively and used without further purification.

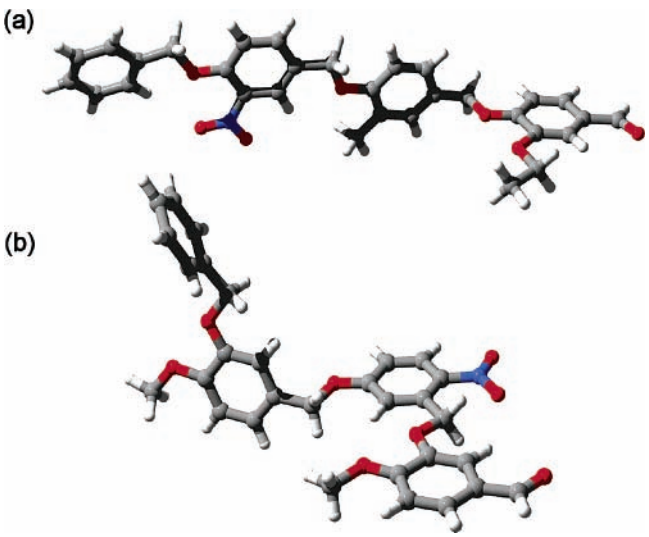

Figure 2. X-ray structure of compounds **43** (a) and **59** (b). Note that the conformations are nonplanar. Ether oligomers are conformationally flexible in solution (NMR).

Most of the ether oligomers obtained were crystalline solids. Structures were confirmed by NMR, MS, and in selected cases by X-ray crystallography (Figure 2).

As for oxime oligomers, we expected that our ether oligomers might be suited to bind to proteins interacting with linear polymers due to the elongated shape of the molecules. The earlier observation of micromolar protease inhibition with our oxime oligomers was consistent with this hypothesis. Indeed, proteases possess channel-like binding pockets and interact with several residues on each side of the scissile peptide bond of their peptide substrates.[7] Ether oligomers and their building blocks were assayed for inhibition of five different proteases (subtilisin, trypsin, chymotrypsin, elastase, papain). The assay was carried out at 10 μM inhibitor concentration in the presence of 8% v/v DMSO as a cosolvent, which was sufficient to ensure good solubility. A significant (>70%) level of inhibition was observed under these conditions with trypsin, α-chymotrypsin, and subtilisin. Inhibition was measured using *N*-succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide (pNA)[8] as a chromogenic substrate for α-chymotrypsin and subtilisin and Tosyl-Gly-Pro-Arg amidomethylcoumarin (AMC)[9] as a fluorogenic substrate for trypsin. According to the nitroanilide and the AMC assays, six inhibitors exhibited protease inhibition with IC_{50} values in the low micromolar range (Figures 3 and 4).

Inhibition is observed only with tetramers and pentamers **25**, **41**, **49**, **50**, **75**, and **76**, while none of the building blocks, dimers, or trimers show any activity. This suggests that

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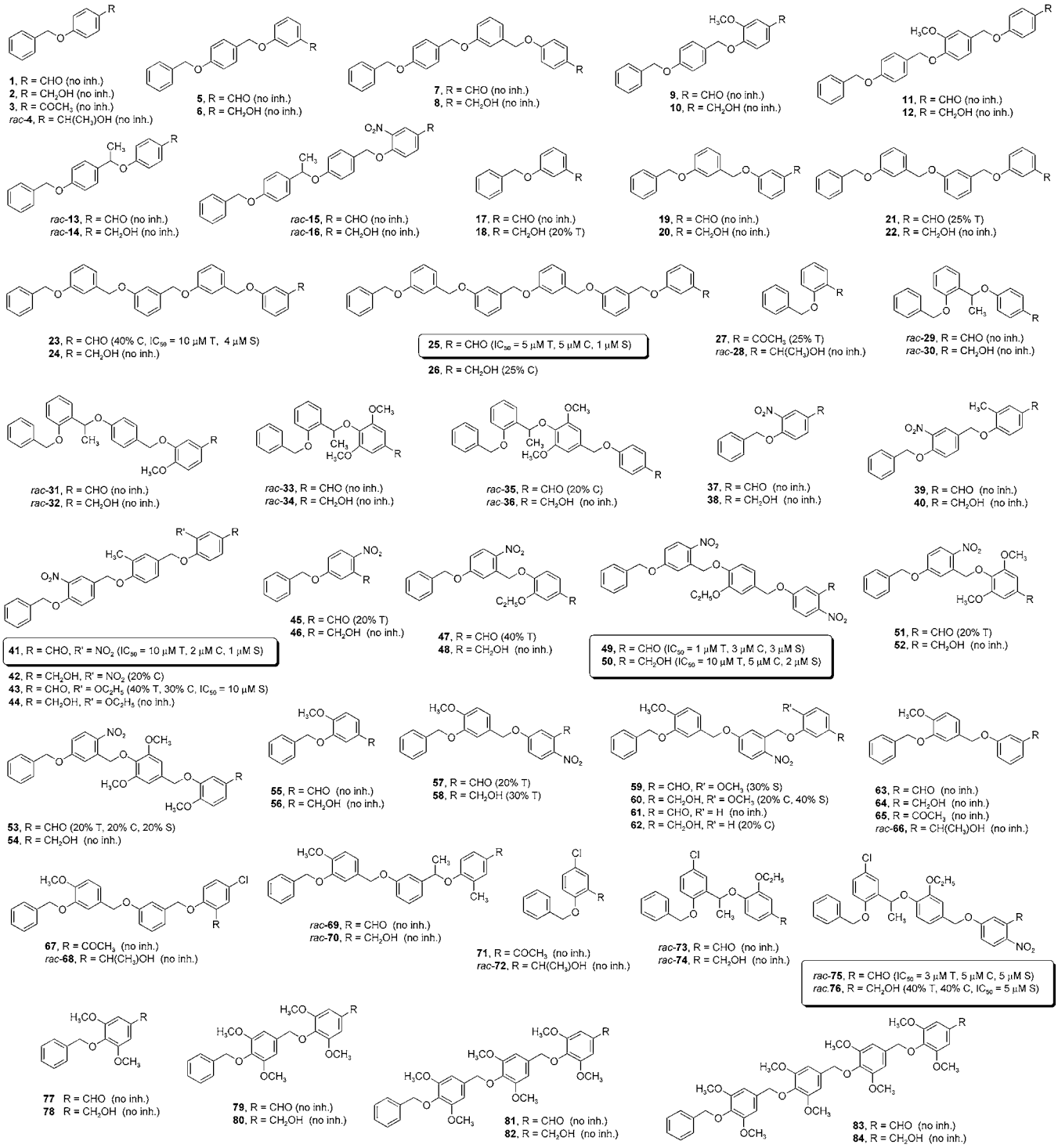

Figure 3. Library of ether oligomers tested for inhibition. Conditions: 10 μM inhibitors, 5 mM aq Bis Tris buffer, pH 7.2, 8% v/v DMSO, 25 °C, and the following enzyme/substrate pairs: 2 μg/mL trypsin and 100 μM Tosyl-Gly-Pro-Arg-AMC; 5 μg/mL α-chymotrypsin and 500 μM *N*-succinyl-Ala-Ala-Pro-Phe-pNA; 5 μg/mL subtilsin and 500 μM *N*-succinyl-Ala-Ala-Pro-Phe-pNA; 20 μg/mL elastase and 500 μM *N*-succinyl-Ala-Ala-Ala-pNA;[10] 50 μg/mL papain in 50 mM phosphate pH 7.2, 2.5 mM EDTA, 1.5 mM DTT, 1 mM *N*-Benzoyl-Arg-pNA.[11] Inhibition was observed only with trypsin (T), α-chymotrypsin (C), and subtilisin (S) and is given in parentheses either as % inhibition in the presence of 10 μM inhibitor or as IC_{50} values for compounds with more than 50% inhibition.

activity is caused by multiple interactions of the inhibitors with the enzymes. All inhibitory oligomers show activities against trypsin, chymotrypsin, and subtilisin but show no activity against either papain or elastase. The compounds showed no detectable inhibition against β-glucosidase and β-galacotidase,[12] providing evidence that the inhibition was

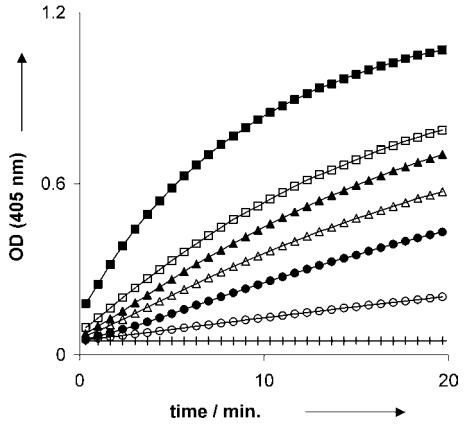

Figure 4. Inhibition of α-chymotrypsin by ether oligomer **25**. Assay conditions: 5 μg/mL α-chymotrypsin (from Bovine Pancreas, Sigma C-7762), 500 μM *N*-succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide (Sigma S-7388), 5 mM aq Bis Tris, pH 7.2, 26 °C, and inhibitor **25** at (●) 0, (●) 3, (●) 6, (△) 9, (●) 12, and (●) 15 or (+) no enzyme.

not caused by nonspecific aggregation.[13] However, and in contrast to our oxime oligomers, the inhibitory ether oligomers did not show any protease inhibition when tested with the bovine serume albumin/calcein sensor system (0.5 mg/mL BSA).[14] This effect might be due to competitive binding of the oligomers to BSA, which possesses binding sites for serum transport of hydrophobic molecules.[15]

Both the cross-reactivity against serine proteases and the interaction with BSA indicate that ether oligomers are less selective than their oxime counterparts. This could be caused by a larger conformational flexibility from the freely rotatable bonds in the ether linkage, allowing for multiple binding conformations. Nevertheless, these somewhat less promising bioactivity properties are outweighed by the much larger flexibility of the synthetic route. Indeed, the ether oligomerization sequence gives higher yields with a much broader variety of hydroxyaromatic building blocks compared to its oxime counterpart. Oxime bond formation was not possible with 2-alkoxy phenols such as anisole and could not be envisioned with ketones due to the formation of *Z*/*E* isomeric mixtures. By contrast, ether bond formation succeeded with almost all building blocks tested irrespective of steric and electronic factors. In addition, the possiblity of using ketones opens the way to the preparation of chiral products.[16]

While only 84 oligomers were prepared here, a total of over 30 000 oligomers are theoretically possible counting dimers, trimers, tetramers, and pentamers from the 13 building blocks used here. The fact that the relative positioning of the two ether attachment points can be varied between different building blocks (*o*-, *m*-, *p*- for the phenol, benzylic, homobenzylic, etc., for the alcohol) implies that the diversity of molecular shapes available from ether oligomers (e.g., Figure 2) is larger than with peptides or peptide-like oligomers bearing side-chain appendages to a regular backbone. Diversity can be further increased by changing the initiating benzylic alcohol. In addition, directed ether bond formation protocols exist for all types of ether bonds (aliphatic−aliphatic, aliphatic−aromatic, aromatic−aromatic),[5,17] such that a very large variety of building blocks are suitable for assembling ether oligomers both in solution and on solid support.[18] It should be possible to adapt this approach to a solid-supported format to construct libraries of diverse ether oligomers for biological testing.

Acknowledgment. This work was supported by the Swiss National Science Foundation and the Novartis Foundation. The X-ray data sets were measured by the BENEFRI Small Molecule Crystallography Service directed by Prof. Helen Stoeckli-Evans.

Supporting Information Available: Spectroscopic data of active compounds **25**, **41**, **49**, **50**, **75**, and **76** and basic crystallographic details of oligomers **43** and **59**. This material is available free of charge via the Internet at http://pubs.acs.org.

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