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Synthesis and Antiviral Activity of Dimeric Capsid-Binding Inhibitors of Human Rhinovirus (HRV)

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A set of dimeric analogues of known rhinovirus capsid-binders Pleconaril **1** and Pirodavir **55** has been synthesized and tested against two representative human rhinovirus (HRV) strains. Dimers with linker lengths ranging from five atoms up to approximately 60 atoms were prepared by coupling various functionalized monomeric precursors. Many of the dimers showed activity against HRV, with the most active compounds being those with the shorter linking groups. The lower activity of all the dimers relative to similar monomeric compounds, and especially the low activity of the longest dimers, suggests that cooperative bivalent binding is not occurring with any of these compounds.

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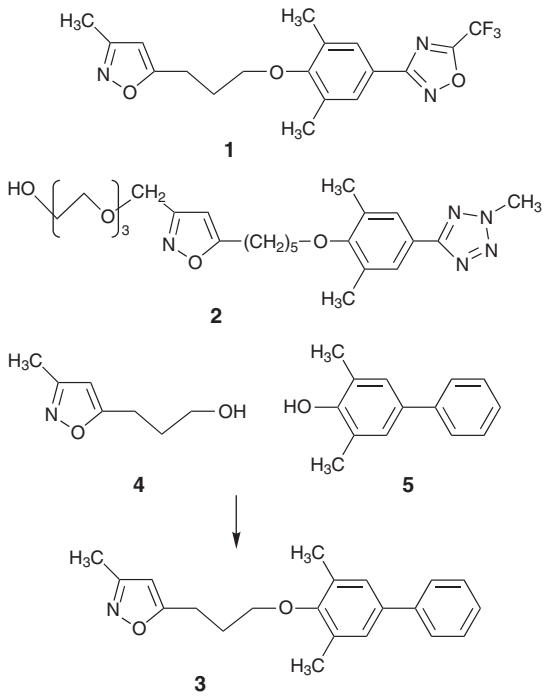
Introduction

Picornaviruses, particularly human rhinoviruses (HRV) cause approximately one-half of all cases of respiratory tract infection (colds)[1] and are responsible for over 25 million physician visits each year in the USA alone.[2] Although HRV infections are generally self limiting, they are also associated with several serious upper and lower respiratory tract complications such as otitis media, chronic bronchitis, and asthma.[3] No effective anti-rhinoviral agent is currently available for the control of HRV, but during the past decade three classes of highly active compounds have been reported including HRV capsid-binding compounds,[4] RNA synthesis inhibitors,[5] and HRV 3C protease inhibitors.[6] The most studied and advanced compound is the capsid-binder Pleconaril **1** (Scheme 1) which has been shown to shorten the duration of upper respiratory illness in two large Phase 3 clinical studies in adults.[7]

From the many solved X-ray structures of various strains of HRV it is known that each virus particle consists of 60 copies of a four-protein subunit or protomer. These protomers form a perfect icosahedral shell or capsid with a diameter of approximately 300 Å, and it has also been well established that within each protein subunit there is a hydrophobic pocket where capsid-binding compounds can reside.[8] Like the HRV protomers, the capsid-binding pockets are arranged into 12 pentameric subunits, with each capsid-binding site separated from its nearest neighbours by approximately 30–40 Å (Fig. 1*a*). In all the solved X-ray structures, these pockets have similar features, often being described as 'foot shaped' and possessing a hydrophobic 'buried toe' and a 'heel region' which is close to an opening or pore region on the protein surface (Fig. 1*b*).[9]



Scheme 1.

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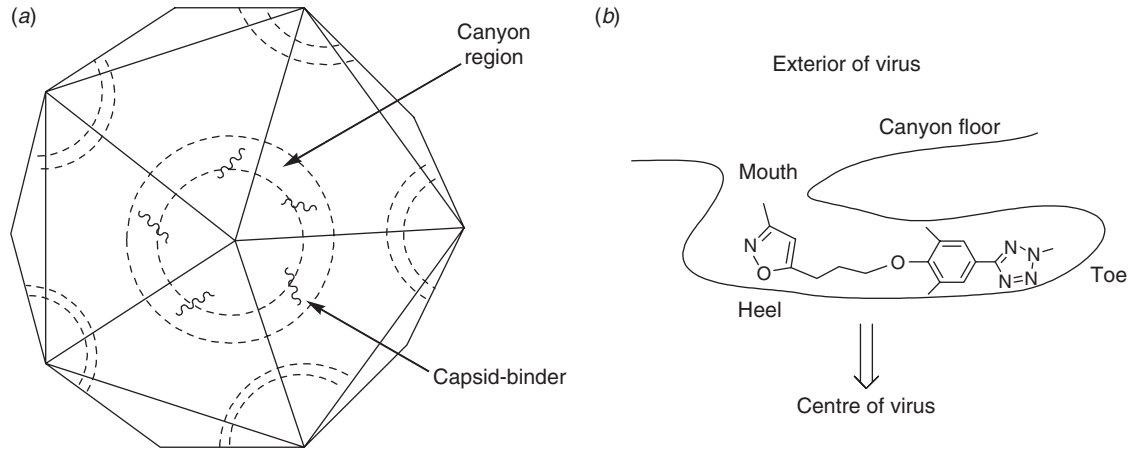


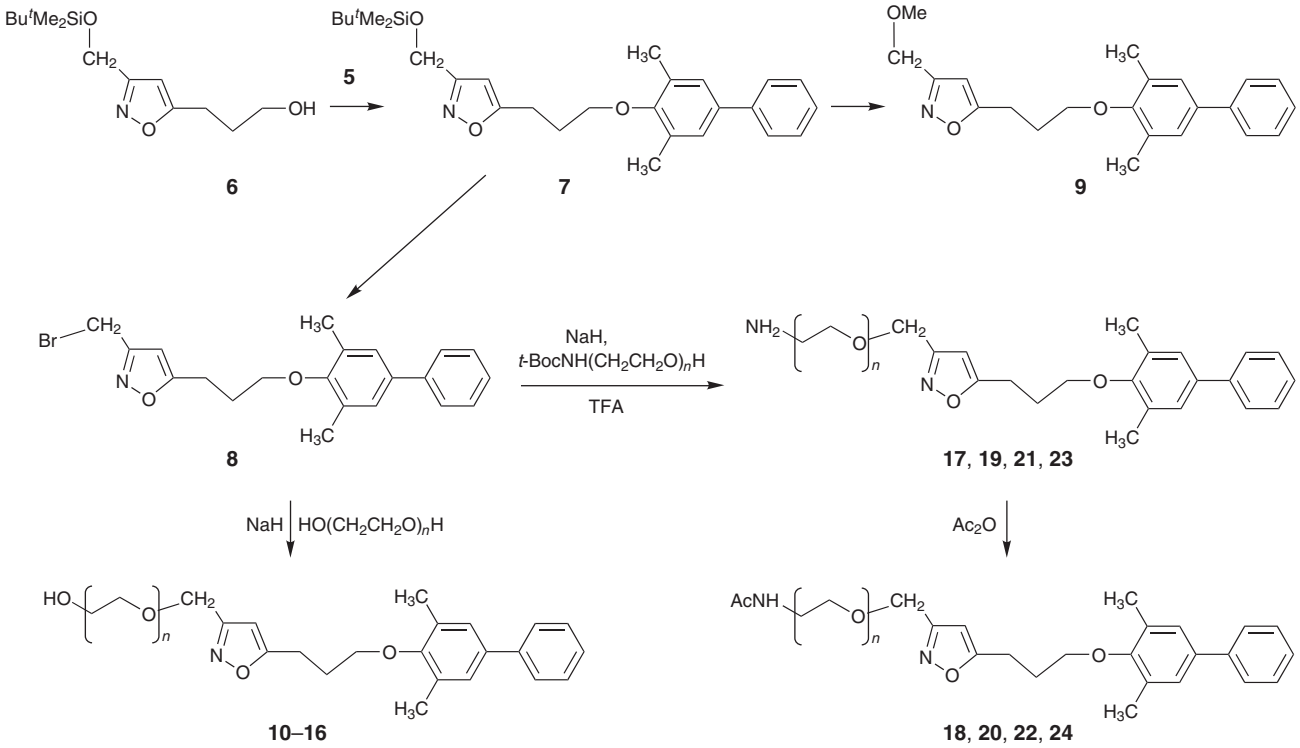
Fig. 1. (*a*) Representation of the HRV capsid showing one fivefold axis of symmetry, the exterior canyon, and the location of capsid-binders. (*b*) Schematic representation of an HRV capsid-binding compound sitting in the hydrophobic pocket beneath the HRV canyon floor.

Multivalent presentation of ligands, such as the carbohydrate groups of a glycoprotein, is often used by nature to form stronger attachments with receptor proteins on the surface of cells, bacteria, or viruses, and synthetic multivalent polymers have also been shown to give extra binding affinity in some systems.[10] There is considerable current interest in the application of multivalent binding to the design of high-affinity bioactive compounds,[11–13] and a variety of compounds have shown improved biological activity when dimerized in a suitable manner.[14] In the search for influenza inhibitors, it has been well established that compounds with two or more appropriately linked sialic acid groups can have vastly improved binding affinity for influenza hemagglutinin,[15,16] although this does not necessarily give predictable in vivo activity.[17] It has also recently been shown that trimeric influenza neuraminidase inhibitors show outstanding antiviral activity.[18] We became interested to explore whether a multivalent form of HRV capsid-binder, and in particular dimeric compounds, would have an increased binding affinity for virus and therefore higher antiviral activity than the analogous monomeric ligand.[19] We considered that a multivalent or bivalent capsid-binder could potentially give rise to several different and new types of antiviral effect including blocking the virus/receptor binding, alteration in the stability of virions, and aggregation of virions.

Results and Discussion

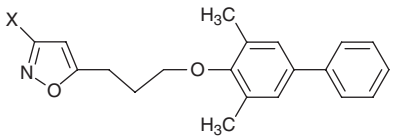
We set out to make dimeric HRV capsid-binders with a range of linking groups, including some with 40 or more atoms in the spacer chain, so as to allow for the possibility that dimers could span adjacent sites on the one virus particle or cause cross-linking and aggregation of multiple virions. Although the direct distance between capsid-binding sites on the same virion is only approximately 30–40 Å, we also needed to take into account that the binding sites are buried approximately 10–15 Å beneath the canyon floor. Given the well described and high anti-HRV activity of Pleconaril **1** and related compounds, we chose to use this class of capsid-binder as our model ligand.[20] In preparing multivalent ligands, it is important to identify a neutral point on the ligand through which a linker can be attached without significant interference to binding. In the case of Pleconaril, we were guided by the published X-ray structural data for co-crystals of HRV with compounds that are closely related to Pleconaril.[21] The X-ray structures show that the isoxazole ring is located at the heel (or mouth) end of the capsid-binding pocket, and we therefore considered that the linking group should be attached at this end of the molecule. In support of this plan, we also noted that there are some earlier reports indicating that analogues of **1** with long polar substituents at the 3-position of the isoxazole ring, such as compound **2**, still ring retain high anti-HRV activity.[22]

As a first approach to the synthesis of dimeric HRV inhibitors, we decided to use derivatives of the biphenyl compound **3** as the monomeric ligand unit on the basis that **3** is known to have high anti-HRV activity, the synthesis of **3** has been well described,[23] and is significantly simpler than that required for Pleconaril **1**. The synthesis of derivatives of compound **3** was carried out following a similar approach to that reported for related structures,[20] and involved the preparation and coupling via Mitsunobu reaction of 5-hydroxypropyl-3-methylisoxazole **4** and 2,6-dimethyl-4-phenylphenol **5** (Scheme 1). To enable attachment of linking groups at the isoxazole 3-position, the *tert*-butyldimethylsilyl (TBDMS) ether protected form of the 3-hydroxymethylisoxazole derivative **6** was prepared[20] and coupled with the phenol **5** to give the functionalized isoxazole–biphenyl derivative **7** (Scheme 2). Removal of the TBDMS protecting group on **7** and conversion of the 3-hydroxymethyl group into the bromomethyl derivative **8** was carried out by standard methods. The methoxymethylisoxazole analogue **9** was also prepared from the hydroxymethylisoxazole derivative **7** for use as a control or benchmark compound in the antiviral assays. The bromomethyl compound **8** was treated with an excess amount of each example of a set of ethylene glycols using sodium hydride as the base to give a set of monomeric ligands **10**–**16** (Table 1) with polyethylene glycol (PEG) tails of varying lengths and including a free terminal hydroxy group to allow dimerization (Scheme 2).



Scheme 2.

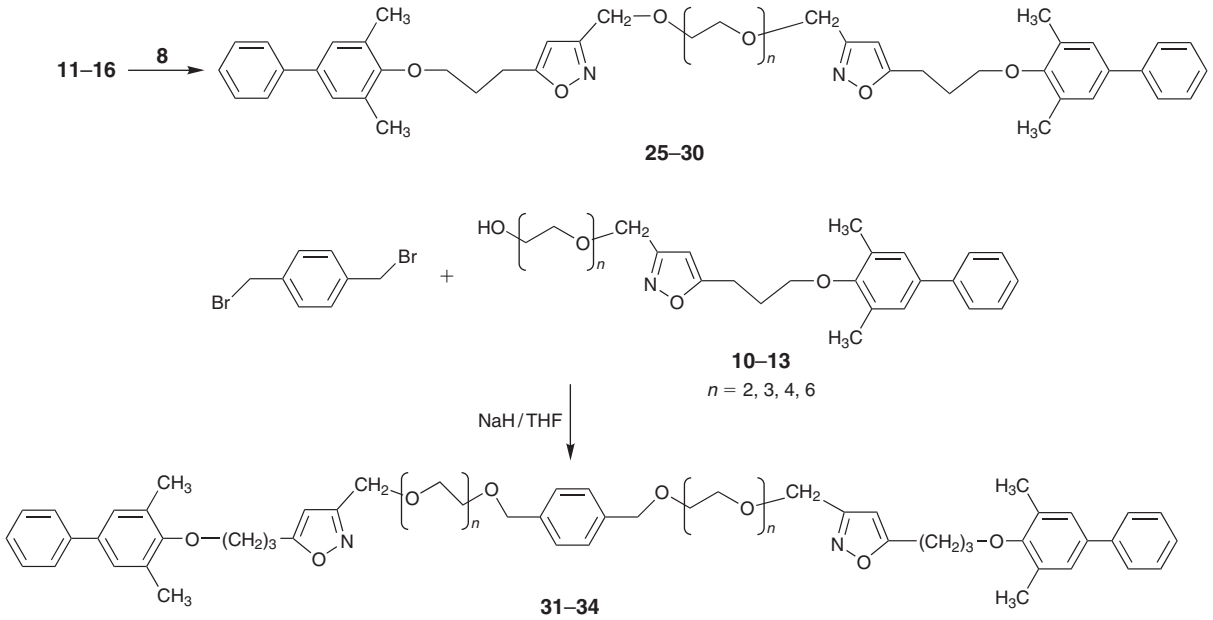
Table 1. Monomeric biphenyl precursors



Compound no.	Isoxazole 3-substituent X	Activity on HRV-1A [IC_{50}, $\mu g\,mL^{-1}$]	Activity on HRV-2 [IC_{50}, $\mu g\,mL^{-1}$]
9	CH_2OCH_3	0.09	<0.05
10	$CH_2OCH_2CH_2OCH_2CH_2OH$	NT	NT
11	$CH_2O(CH_2CH_2O)_2CH_2CH_2OH$	0.38	<0.05
12	$CH_2O(CH_2CH_2O)_3CH_2CH_2OH$	1.5	0.2
13	$CH_2O(CH_2CH_2O)_5CH_2CH_2OH$	>50	0.1
14	$CH_2O(CH_2CH_2O)_7CH_2CH_2OH$[A]	>50	<0.05
15	$CH_2O(CH_2CH_2O)_{10}CH_2CH_2OH$[A]	>50	1
16	$CH_2O(CH_2CH_2O)_{19}CH_2CH_2OH$[A]	>50	14
17	$CH_2OCH_2CH_2OCH_2CH_2NH_2$	NT[B]	<0.05
18	$CH_2OCH_2CH_2OCH_2CH_2NHAc$	0.85	<0.05
19	$CH_2O(CH_2CH_2O)_2CH_2CH_2NH_2$	NT	<0.05
20	$CH_2O(CH_2CH_2O)_2CH_2CH_2NHAc$	1.2	0.05
21	$CH_2O(CH_2CH_2O)_3CH_2CH_2NH_2$	NT	1
22	$CH_2O(CH_2CH_2O)_3CH_2CH_2NHAc$	>50	0.2
23	$CH_2O(CH_2CH_2O)_5CH_2CH_2NH_2$	NT	2
24	$CH_2O(CH_2CH_2O)_5CH_2CH_2NHAc$	>50	0.06

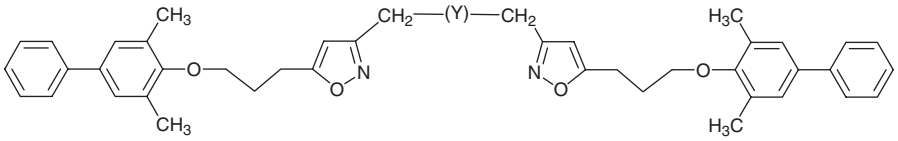
[A] Compounds **14**, **15**, and **16** were prepared from PEG mixtures of narrow distribution. The number shown is the mean number of glycol units.
[B] In Tables 1–4, NT means not tested.

While compounds **10**–**13** were prepared as discrete compounds, the longer-length glycols actually comprised PEG of a narrow molecular weight range, and thus compounds **14**–**16** consisted of a mixture of several PEG derivatives of varying length, e.g. $RO–(CH_2CH_2O)_nCH_2CH_2OH$, where $n+1$ is the average value of the number of PEG units. The bromomethyl compound **8** was also treated with a set of *t*-Boc-protected N-terminal ethylene glycols (Scheme 2)



Scheme 3.

Table 2. Dimeric biphenyl compounds

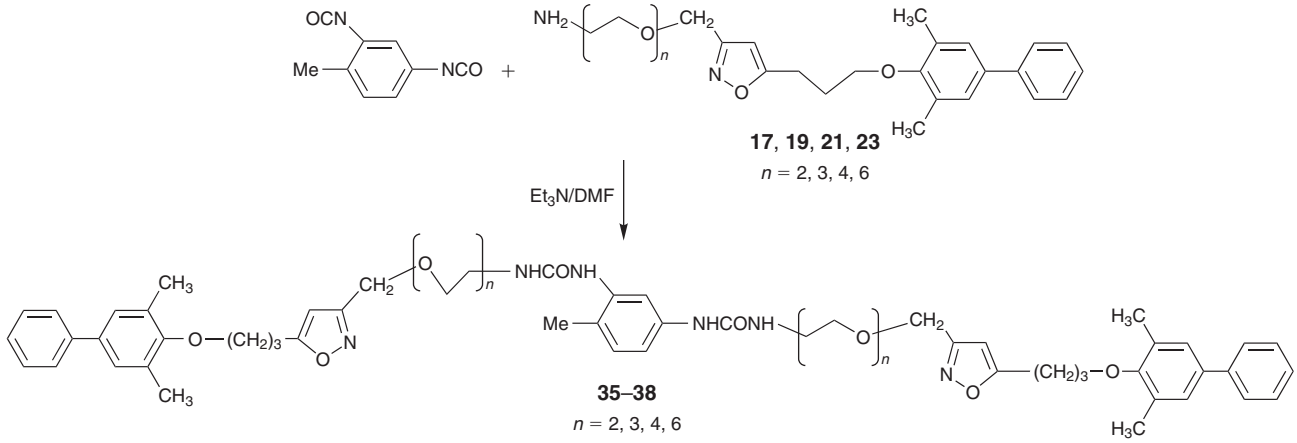


Compound no.	Central linking group Y	Activity on HRV-1A [IC_{50}, $\mu g\,mL^{-1}$]	Activity on HRV-2 [IC_{50}, $\mu g\,mL^{-1}$]
1	Pleconaril (monomer)	0.07	<0.05
9	Monomer	0.07	<0.05
25	$O(CH_2CH_2O)_2CH_2CH_2O$	0.32	0.1
26	$O(CH_2CH_2O)_3CH_2CH_2O$	0.76	0.06
27	$O(CH_2CH_2O)_5CH_2CH_2O$	>50	0.03
28	$O(CH_2CH_2O)_7CH_2CH_2O$[A]	NT	<0.05
29	$O(CH_2CH_2O)_{10}CH_2CH_2O$[A]	NT	0.1
30	$O(CH_2CH_2O)_{19}CH_2CH_2O$[A]	NT	1
31	$O(CH_2CH_2O)_2CH_2$–C_6H_4–$CH_2(OCH_2CH_2)_2O$	0.39	0.1
32	$O(CH_2CH_2O)_3CH_2$–C_6H_4–$CH_2(OCH_2CH_2)_3O$	0.28	<0.05
33	$O(CH_2CH_2O)_4CH_2$–C_6H_4–$CH_2(OCH_2CH_2)_4O$	>50	0.2
34	$O(CH_2CH_2O)_6CH_2$–C_6H_4–$CH_2(OCH_2CH_2)_6O$	>50	0.2
35	$(OCH_2CH_2)_2NHCONHC_6H_3(Me)NHCONH(CH_2CH_2O)_2$	>50	0.4
36	$(OCH_2CH_2)_3NHCONHC_6H_3(Me)NHCONH(CH_2CH_2O)_3$	>50	0.3
37	$(OCH_2CH_2)_4NHCONHC_6H_3(Me)NHCONH(CH_2CH_2O)_4$	>50	0.3
38	$(OCH_2CH_2)_6NHCONHC_6H_3(Me)NHCONH(CH_2CH_2O)_6$	>50	0.02

[A] Compounds **28**, **29**, and **30** were prepared from PEG mixtures of narrow distribution. The number shown is the mean number of glycol units.

to give monomeric amino compounds **17**, **19**, **21**, and **23** (Table 1), from which were made the *N*-acetyl derivatives **18**, **20**, **22**, and **24**.

Preparation of dimeric derivatives from the ethylene glycol monomers **10**–**16** was achieved in two different ways (Scheme 3). First, the monomers **11**–**16** were each treated with sodium hydride and then allowed to react with the bromomethyl compound **8** giving a set of dimers **25**–**30** as shown in Table 2. Second, the shorter monomers **10**–**13** were each treated with sodium hydride in tetrahydrofuran and then allowed to react with half an equivalent of α, α′-dibromo-*p*-xylene to give dimers **31**–**34**. The N-terminal monomers **17**, **19**, **21**, and **23** were dissolved in dimethylformamide and treated with half an equivalent of toluene-2,4-diisocyanate to give dimers **35**–**38** (Table 2) as shown in Scheme 4. All of the dimeric biphenyl derivatives **25**–**38** in Table 2 were purified



Scheme 4.

by flash chromatography and characterized from their proton NMR and high-resolution mass spectra.

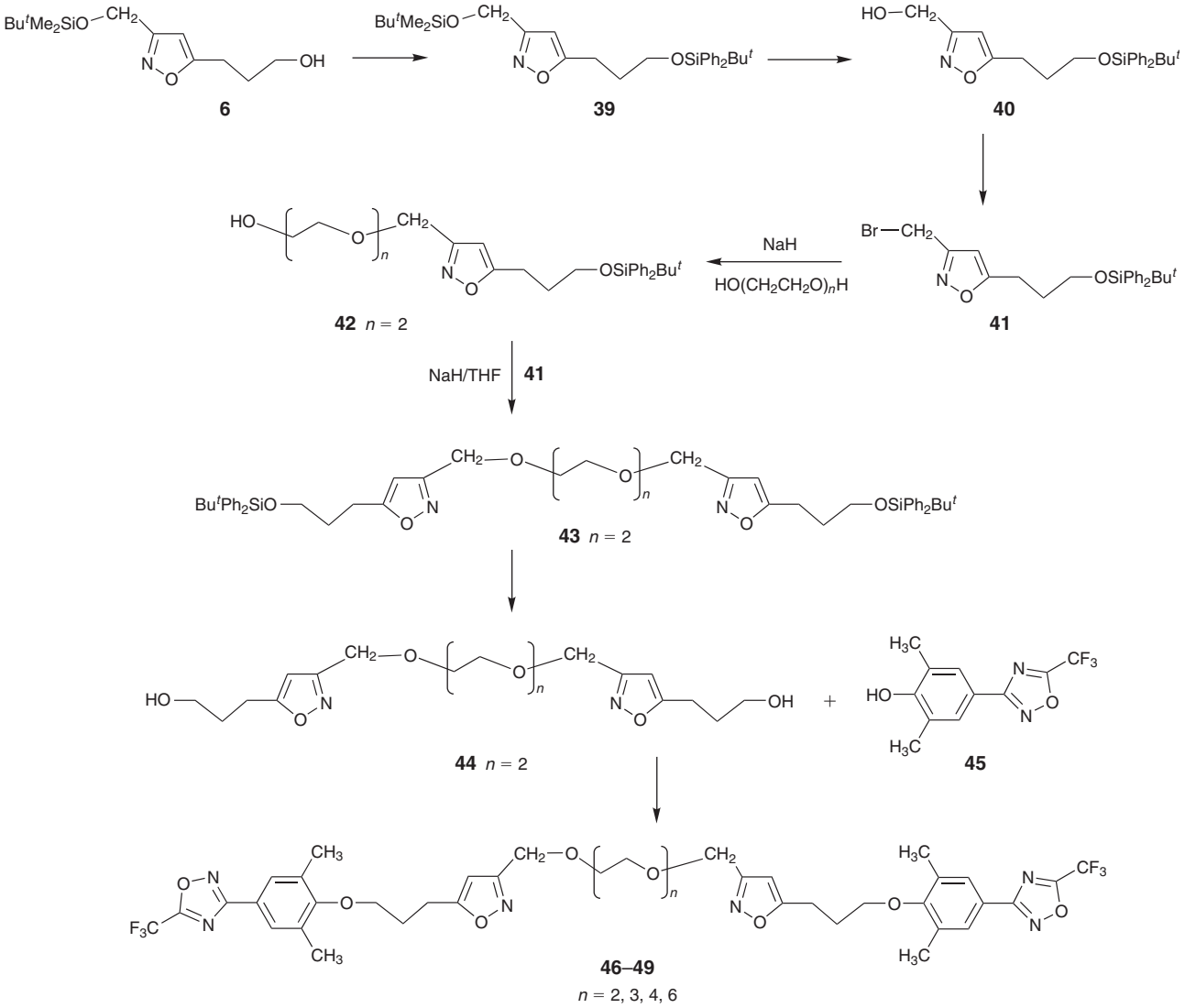
The dimeric biphenyl compounds **25**–**38**, and also most of the monomeric precursor compounds **9**–**24**, were tested for their activity against two strains of HRV using a standard cytopathic effect (CPE) assay,[24] and the results are shown in Tables 1 and 2. The data for the monomeric compounds clearly show that the activity falls off as the length of the isoxazolyl 3-substituent increases. Thus, the simple methoxymethylisoxazole derivative **9** is highly active, but the PEG derivatives are all less active, and compound **16**, which has the longest PEG tail, is virtually inactive on both strains of virus. A similar trend is seen with the N-terminal monomeric derivatives **17**–**24**. The data for the dimeric compounds are less clear-cut, but again there appears to be a general drop in activity as the length of the linking group increases, and none of the dimers is as active as the monomeric control compounds **1** and **9**. We had hoped that we might observe better activity with the longest dimers, particularly compound **30**, as a result of the ability of each end of the molecules to bind simultaneously to neighbouring capsid-binding pockets. Thus, compound **30**, the dimer with the longest linker, has a chain of 60 ± 15 atoms in the PEG bridging group which, if fully extended, could potentially span a distance of approximately 75 Å. In theory, this should be long enough to allow both ends of the molecule to bind simultaneously to neighbouring HRV pockets on the same capsid or to bind to pockets on two different virions, but the low activity results suggest that this is probably not occurring.

Although the anti-viral activity of the biphenyl dimers was disappointing, by the time we received the results of the biological testing, the synthesis of a series of Pleconaril dimers was well under way, so we decided to complete the syntheses and test the dimers. It was found that a different synthetic route was required because when a similar route to the the biphenyl compounds was attempted it was observed that decomposition of the 1,2,4-oxadiazole ring occurred during the coupling of the PEG linkers. The alternative route is shown in Scheme 5, and involved coupling of the bromomethylisoxazole derivative **41** with an isoxazole–PEG derivative such as **42** to give a dimeric intermediate such as **43**. Removal of the *tert*-butyldiphenylsilyl groups to give bis(hydroxypropylisoxazole) derivatives such as **44** then allowed addition, using a Mitsunobu reaction, of the 1,2,4-oxadiazolylphenol derivative **45**[25] to give dimers **46**–**49** as the final step. Pleconaril dimers **51**–**54**, which have a central benzyl group, were prepared as outlined in Scheme 6 using the addition of PEG derivatives, such as compound **42**, to α, α'-dibromo-*p*-xylene and then addition of the 1,2,4-oxadiazolylphenol **45**.

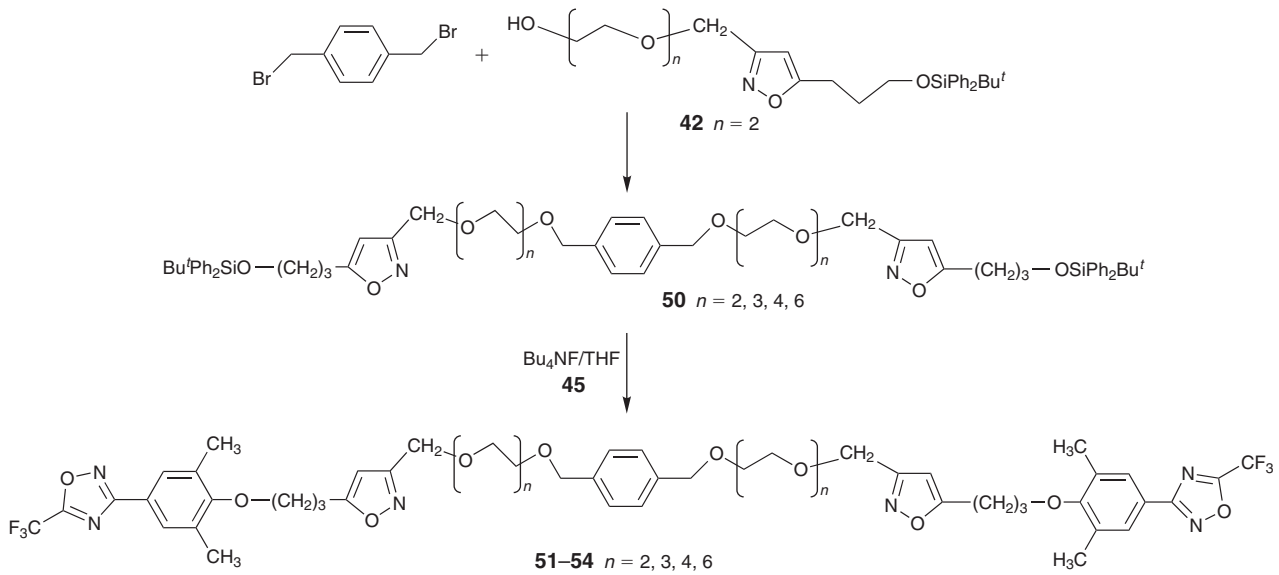
The dimers **46**–**54** were also tested on two representative HRV strains using a standard CPE assay and the results, together with results for Pleconaril, are shown in Table 3. As with the biphenyl dimers in Table 2, none of the dimers **46**–**54** was as active as the monomeric Pleconaril and, if anything, the activity appeared to gradually decline with increasing linker length. Two representative Pleconaril dimers, compounds **46** and **53**, were tested on a wider range of Picornaviruses and the results, as shown in Table 4, confirm that these dimers have a similar spectrum of activity to Pleconaril, but are less potent. Compound **54**, the longest Pleconaril dimer, has a chain of 44 atoms in the linking group which, if fully extended, could potentially span a distance of approximately 50 Å. In theory, this should be long enough to allow both ends of the molecule to bind simultaneously to hydrophobic pockets on two different virions causing aggregation and immobilization of the HRV. However, the low activity results again suggest that this extra mode of binding is probably not occurring.

We have also prepared a single example of a dimeric derivative of another other type of capsid-binder. Thus, we have recently reported that the oxime ether compound **57**[26] in Scheme 7 is a highly active analogue of the known capsid-binder Pirodavir **55**. By reaction of two equivalents of the aldehyde precursor **56** with the bis(alkoxyamine) derivative **58** of tetraethylene glycol, we made the dimeric oxime ether analogue **59**. Compound **59** was tested on HRV-2 and HRV-14, but did not show any significant activity.

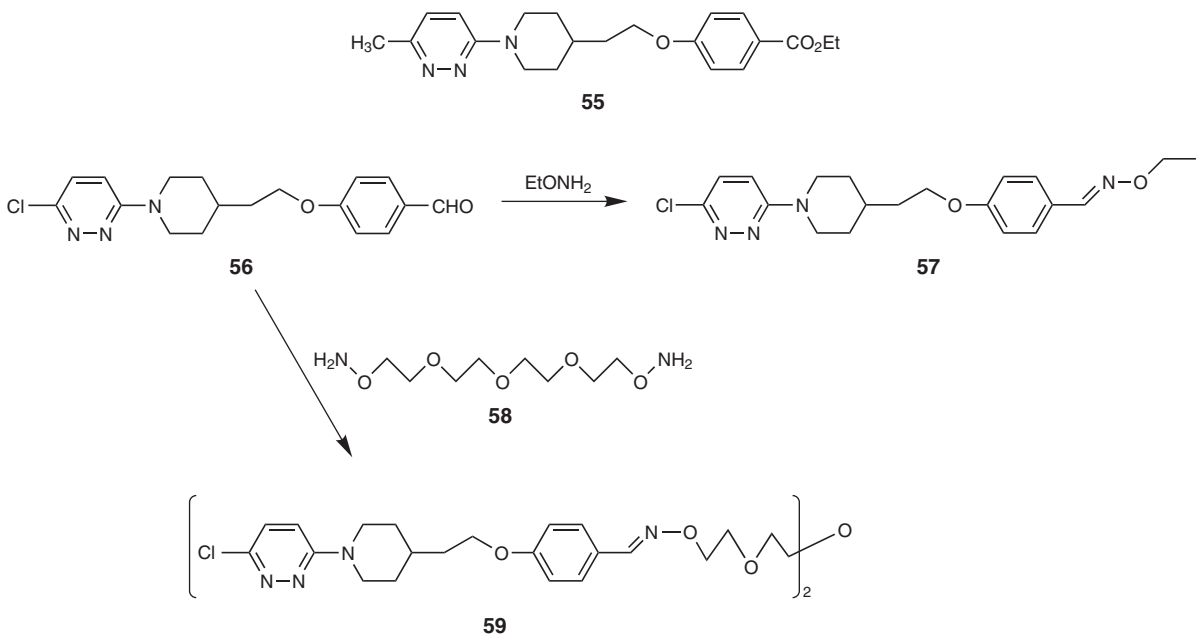
There could be several reasons why bivalent binding of the dimeric compounds does not appear to take place, including unfavourable interactions between the linker group and the viral capsid, and the large entropy cost involved in getting the second binding event to occur. The capsid-binding pocket is



Scheme 5.

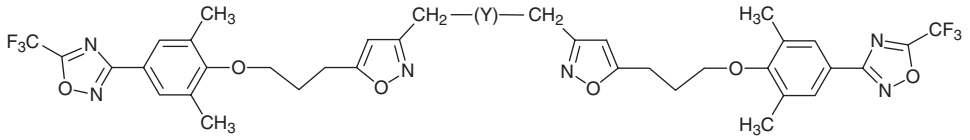


Scheme 6.



Scheme 7.

Table 3. Dimeric pleconaril derivatives



Compound no.	Central linking group Y	Activity on HRV-1A [IC_{50}, $\mu g\,mL^{-1}$][A]	Activity on HRV-2 [IC_{50}, $\mu g\,mL^{-1}$]
1	Pleconaril (monomer)	0.02	0.03
46	$OCH_2CH_2OCH_2CH_2O$	0.06	0.15
47	$O(CH_2CH_2O)_2CH_2CH_2O$	0.56	0.1
48	$O(CH_2CH_2O)_3CH_2CH_2O$	0.57	0.13
49	$O(CH_2CH_2O)_5CH_2CH_2O$	>10	0.04
51	$O(CH_2CH_2O)_2CH_2–C_6H_4–CH_2(OCH_2CH_2)_2O$	NT	0.12
52	$O(CH_2CH_2O)_3CH_2–C_6H_4–CH_2(OCH_2CH_2)_3O$	>10	0.2
53	$O(CH_2CH_2O)_4CH_2–C_6H_4–CH_2(OCH_2CH_2)_4O$	1.8	0.15
54	$O(CH_2CH_2O)_6CH_2–C_6H_4–CH_2(OCH_2CH_2)_6O$	>10	0.15

[A] None of the compounds showed any cellular toxicity at 10 $\mu g\,mL^{-1}$.

Table 4. Activity of representative dimers on other picornaviruses

Picornavirus type	Activity [IC_{50}, $\mu g\,mL^{-1}$]		
	Dimer **46**	Dimer **53**	Pleconaril **1**
Coxsackie-A21	0.35	0.36	0.003
Coxsackie-B3	>50	9.6	1
Echo-21	0.0006	0.0026	NT
Enterovirus-70	>50	2.88	0.28
HRV-14	0.016	0.05	0.01
Polio-2	>50	40	>50

certainly more deeply buried than a typical enzyme active site, and the first step of a dimer-binding event requires one end of a rather large and long ligand to enter at the fairly narrow mouth of a binding pocket. On the other hand, a monomeric capsid-binder has several advantages over the dimers including the potential to enter the binding site from either end, to fit entirely within the binding site, and to be completely enveloped by the protein. These factors together may account for the higher activity of the simple monomers.

Conclusions

We have prepared derivatives of Pleconaril **1**, and the related biphenyl analogue **3**, in which the methyl substituent on the isoxazole ring is functionalized to allow the attachment of ethylene glycol linking groups. Thus a series of symmetrical ethylene glycol-linked dimeric compounds has been prepared and tested for anti-HRV activity. The dimers show generally lower activity than monomers **1** and **9**, a result suggesting that,

although they are probably binding to the same hydrophobic pocket as Pleconaril, only one end of each dimer is able to bind at a time and the second ligand group provides no enhancement to the binding affinity or antiviral activity.

Experimental

Thin-layer chromatography (TLC) was performed on E. Merck Kieselgel 60 F-254 plates. Flash chromatography was carried out routinely using Merck silica gel 60, 230–400 mesh, and compounds were isolated as pure materials as confirmed by the presence of a single spot on TLC. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 300 MHz on a Bruker DPX-300 spectrometer. The ^{1}H NMR spectroscopic data refer to deuterated chloroform solutions ($CDCl_3$) unless otherwise indicated, and chemical shifts (δ) were calibrated against the residual solvent peak. High-resolution mass spectra for accurate mass determinations were recorded on a Bruker BioApex 47e Fourier-transform mass spectrometer fitted with an Analytica electrospray source. Low-resolution mass spectra were recorded on a VG micromass 70/70F or a VG TRIO-1 mass spectrometer with an ion source temperature of 200°C and electron impact energy of 70 eV.

Pleconaril ***1***

5-(Trifluoromethyl)-3-[3,5-dimethyl-4-{[3-(3-methyl-5-isoxazolyl)propyl]oxy}phenyl]-1,2,4-oxadiazole (Pleconaril) **1** was prepared following the method described in the literature.[25] The compound was purified by chromatography on silica gel to give a colourless low-melting solid; NMR and mass spectra were consistent with the reported values.

3-(Bromomethyl)-5-[3-(2,6-dimethyl-4-phenylphenoxy)-propyl]isoxazole ***8***

3-(Hydroxymethyl)-5-[3-(2,6-dimethyl-4-phenylphenoxy)propyl]isoxazole was prepared following procedures described in the literature for very similar compounds.[20] Thus 3-(*tert*-butyldimethylsilyloxymethyl)-5-(3-hydroxypropyl)isoxazole **6**[20] and 2,6-dimethyl-4-phenyl-phenol **5** were coupled by way of a Mitsunobu reaction to give the adduct, 3-(*tert*-butyldimethylsilyloxymethyl)-5-[3-(2,6-dimethyl-4-phenyl-phenoxy)propyl]isoxazole **7**, in 82% yield.

Removal of the silyloxy group under acidic hydrolysis gave the hydroxy compound, *5-[3-(2,6-dimethyl-4-phenylphenoxy)propyl]-3-(hydroxymethyl)isoxazole*, in 93% yield. δ_H 7.6–7.2 (m, 7H), 6.13 (s, 1H), 4.75 (d, 2H), 3.86 (t, 2H), 3.07 (t, 2H), 2.33 (s, 6H), 2.23 (m, 2H), 2.1 (t, OH). *m*/*z* (ES) 338.1748 $(M+H)^+$. Calc. for $C_{21}H_{24}NO_3$: 338.1750. Bromination of the hydroxy compound using the general literature procedure[22] gave the *bromomethyl compound* **8** in 95% yield. δ_H 7.6–7.2 (m, 7H), 6.17 (s, 1H), 4.41 (s, 2H), 3.86 (t, 2H), 3.07 (t, 2H), 2.33 (s, 6H), 2.23 (m, 2H). *m*/*z* (ES) 422.0725 $(M+Na)^+$. Calc. for $C_{21}H_{22}BrNNaO_2$: 422.0720.

5-[3-(2,6-Dimethyl-4-phenylphenoxy)propyl]-3-(methoxymethyl)-isoxazole ***9***

Sodium hydride (9 mg, 0.22 mmol) was added to a solution of 5-[3-(2,6-dimethyl-4-phenylphenoxy)propyl]-3-(hydroxymethyl)isoxazole (50 mg, 0.15 mmol) in THF (3 mL) at 0°C, and then the reaction mixture was allowed to warm to room temperature and stirred for 1 h under argon. Methyl iodide (105 mg, 0.74 mmol) was added and reaction mixture was stirred overnight. Water (1 mL) was added and the mixture was partitioned between ethyl acetate (50 mL) and water (10 mL); the organic phase was washed with brine, dried (Na_2SO_4), and concentrated. Chromatography of the crude product on silica gel (10 g) using as eluent 85 : 15 hexane/ethyl acetate gave compound **9** in 100% yield. δ_H 7.6–7.2 (m, 7H), 6.12 (s, 1H), 4.51 (s, 2H), 3.87 (t, 2H), 3.40 (s, 3H), 3.07 (t, 2H), 2.33 (s, 6H), 2.25 (m, 2H). *m*/*z* (ES) 374 $(M+Na)^+$.

5-[3-(2,6-Dimethyl-4-phenylphenoxy)propyl]-3-(2-hydroxyethoxy)-ethoxymethylisoxazole ***10***

A mixture of sodium hydride (60% in oil, 7.5 mg, 187 μmol) and diethylene glycol (45 mg, 425 μmol) was stirred in THF (2 mL) under argon for 1 h, and then tetrabutylammonium iodide (5 mg) and a solution of bromomethyl compound **8** (75 mg, 187 μmol) in THF (1.5 mL) were added; the reaction mixture was allowed to stir overnight. After addition of saturated ammonium chloride (1 mL), the reaction mixture was partitioned between ethyl acetate (50 mL) and water (10 mL). The organic phase was washed with brine (15 mL), dried (Na_2SO_4), and then concentrated to give a pale yellow oil. The crude product was chromatographed on silica gel (12 g), eluent 1 : 1 ethyl acetate/hexanes, to give the *title compound* **10** (72 mg, 61%) as a colourless oil. δ_H 7.6–7.2 (m, 7H), 6.15 (s, 1H), 4.64 (s, 2H), 3.86 (t, 2H), 3.8–3.6 (m, 8H), 3.07 (t, 2H), 2.33 (s, 6H), 2.23 (m, 2H). *m*/*z* (ES) 448.2082 $(M+Na)^+$. Calc. for $C_{25}H_{31}NNaO_5$: 448.2100.

General Procedure for the Preparation of 5-[3-(2,6-Dimethyl-4-phenylphenoxy)propyl]-3-[hydroxy(ethyleneoxy)$_n$methyl]-isoxazoles ***11****–****16***

Compounds **11**–**16** were prepared from reaction of the bromomethyl compound **8** with the appropriate ethylene glycol and sodium hydride using essentially the same method as described for the preparation of compound **10**. The compounds were purified on silica gel and characterized by their NMR spectra and mass spectrometric data.

Compound **11**: δ_H 7.6–7.2 (m, 7H), 6.17 (s, 1H), 4.63 (s, 2H), 3.86 (t, 2H), 3.8–3.6 (m, 12H), 3.06 (t, 2H), 2.33 (s, 6H), 2.23 (m, 2H). *m*/*z* (ES) 492.2384 $(M+Na)^+$. Calc. for $C_{27}H_{35}NNaO_6$: 492.2362.

Compound **12**: δ_H 7.6–7.2 (m, 7H), 6.16 (s, 1H), 4.62 (s, 2H), 3.86 (t, 2H), 3.66 (m, 16H), 3.06 (t, 2H), 2.32 (s, 6H), 2.22 (m, 2H). *m*/*z* (ES) 536.2609 $(M+Na)^+$. Calc. for $C_{29}H_{39}NNaO_7$: 536.2624.

Compound **13**: δ_H 7.6–7.2 (m, 7H), 6.14 (s, 1H), 4.61 (s, 2H), 3.86 (t, 2H), 3.65 (m, 24H), 3.06 (t, 2H), 2.33 (s, 6H), 2.22 (m, 2H). *m*/*z* (ES) 624.3134 $(M+Na)^+$. Calc. for $C_{33}H_{47}NNaO_9$: 624.3149.

Compound **14**: δ_H 7.6–7.2 (m, 7H), 6.14 (s, 1H), 4.61 (s, 2H), 3.87 (t, 2H), 3.75–3.6 (m, 31H), 3.06 (t, 2H), 2.32 (s, 6H), 2.22 (m, 2H). *m*/*z* (ES) 756.3890 $(M+Na)^+$. Calc. for $C_{39}H_{59}NNaO_{12}$: 756.3935.

Compound **15**: δ_H 7.6–7.2 (m, 7H), 6.14 (s, 1H), 4.61 (s, 2H), 3.86 (t, 2H), 3.75–3.6 (m, 34H), 3.06 (t, 2H), 2.32 (s, 6H), 2.22 (m, 2H). *m*/*z* (ES) 932.4922 $(M+Na)^+$. Calc. for $C_{47}H_{75}NNaO_{16}$: 932.4984.

Compound **16**: δ_H 7.6–7.2 (m, 7H), 6.14 (s, 1H), 4.61 (s, 2H), 3.86 (t, 2H), 3.75–3.6 (m, 87H), 3.05 (t, 2H), 2.32 (s, 6H), 2.22 (m, 2H). *m*/*z* (ES) 1328.7350 $(M+Na)^+$. Calc. for $C_{65}H_{111}NNaO_{25}$: 1328.7343.

3-(2-Aminoethoxy)ethoxymethyl-5-[3-(2,6-dimethyl-4-phenylphenoxy)propyl]isoxazole ***17***

Reaction of the bromomethyl compound **8** with 2-(2-aminoethoxy-*N*-*tert*-butyloxycarbonyl)ethanol[27] using essentially the same method as described for **10** gave the adduct, 3-(2-aminoethoxy-*N*-*tert*-butyloxycarbonyl)ethoxymethyl-5-[3-(2,6-dimethyl-4-phenylphenoxy)propyl]isoxazole in 91% yield. Trifluoroacetic acid (1 mL) was added to a solution of the adduct (240 mg, 0.46 mmol) in CH_2Cl_2 (10 mL), and the reaction mixture was allowed to stir under argon for 2 h. The mixture was concentrated under vacuum, and then the crude product was partitioned between brine/sodium bicarbonate (1 : 1, 20 mL) and ethyl acetate (2 × 100 mL). The combined organic phase was dried (Na_2SO_4) and concentrated, the crude product was chromatographed on silica gel (20 g), eluent 92.5 : 7.5 CH_2Cl_2/10% ammonia in methanol, to give the *amino compound* **17** as a colourless oil (71%). δ_H (CD_3OD) 7.5–7.15 (m, 7H), 6.24 (s, 1H), 4.55 (s, 2H), 3.81 (t, 2H), 3.61 (s, 4H), 3.46 (t, 2H), 3.03 (t, 2H), 2.72 (t, 2H), 2.26 (s, 6H), 2.16 (m, 2H). *m*/*z* (ES) 425.2428 $(M+H)^+$. Calc. for $C_{25}H_{33}N_2O_4$: 425.2432.

*3-(2-*N*-Acetylaminoethoxy)ethoxymethyl-5-[3-(2,6-dimethyl-4-phenylphenoxy)propyl]isoxazole* ***18***

Acetic anhydride (67 mg, 0.66 mmol) was added to a solution of compound **17** (28 mg, 66 μmol) in pyridine (1.5 mL), and the reaction mixture was allowed to stir at room temperature for 4 days under an atmosphere of argon. The solvents were removed under vacuum and

the crude residue was chromatographed on silica gel (10 g), eluent 96 : 4 dichloromethane/methanol, to give the N-*acetamido compound* **18** (27 mg, 88%) as a colourless oil. δ_H 7.6–7.2 (m, 7H), 6.13 (s, 1H), 4.64 (s, 2H), 3.87 (t, 2H), 3.65 (s, 4H), 3.56 (m, 2H), 3.03 (t, 2H), 2.72 (t, 2H), 2.26 (s, 6H), 2.16 (m, 2H). *m/z* (ES) 489.2388 $(M + Na)^+$. Calc. for $C_{27}H_{34}N_2NaO_5$: 489.2351.

*General Procedure for the Preparation of 3-[(Aminoethoxy)-(ethyleneoxy)$_n$methyl]-5-[3-(2,6-dimethyl-4-phenylphenoxy)-propyl]isoxazoles **19**, **21**, and **23***

Compounds **19**, **21**, and **23** were prepared from reaction of the bromomethyl compound **8** with the appropriate *tert*-butoxycarbonylamino-ethoxy–ethylene glycol[28] and sodium hydride using essentially the same method as described for the preparation of compound **17**. The compounds were purified on silica gel and characterized by their NMR spectra and mass spectrometric data.

Compound **19**: δ_H (CD_3OD) 7.6–7.2 (m, 7H), 6.28 (s, 1H), 4.58 (s, 2H), 3.84 (t, 2H), 3.6 (m, 8H), 3.49 (t, 2H), 3.06 (t, 2H), 2.75 (br, 2H), 2.29 (s, 6H), 2.19 (m, 2H). *m/z* (ES) 469.2718 $(M + H)^+$. Calc. for $C_{27}H_{37}N_2O_5$: 469.2702.

Compound **21**: δ_H (CD_3OD) 7.6–7.2 (m, 7H), 6.28 (s, 1H), 4.58 (s, 2H), 3.85 (t, 2H), 3.7–3.55 (m, 12H), 3.49 (t, 2H), 3.07 (t, 2H), 2.75 (br, 2H), 2.30 (s, 6H), 2.20 (m, 2H). *m/z* (ES) 513.2949 $(M + H)^+$. Calc. for $C_{29}H_{41}N_2O_6$: 513.2965.

Compound **23**: δ_H (CD_3OD) 7.6–7.2 (m, 7H), 6.28 (s, 1H), 4.59 (s, 2H), 3.84 (t, 2H), 3.7–3.5 (m, 22H), 3.06 (t, 2H), 2.93 (br, 2H), 2.29 (s, 6H), 2.20 (m, 2H). *m/z* (ES) 601.3471 $(M + H)^+$. Calc. for $C_{33}H_{49}N_2O_8$: 601.3489.

*General Procedure for the Preparation of 3-[2-*N*-Acetylamino-(ethyleneoxy)$_n$methyl]-5-[3-(2,6-dimethyl-4-phenylphenoxy)-propyl]isoxazoles **20**, **22**, and **24***

Compounds **20**, **22**, and **24** were prepared by reaction of the amino compounds **19**, **21**, and **23** with acetic anhydride using the same method as described for the preparation of compound **18**. The compounds were purified on silica gel and characterized by their NMR spectra and mass spectroscopic data.

Compound **20**: δ_H 7.6–7.2 (m, 7H), 6.4 (NH), 6.13 (s, 1H), 4.64 (s, 2H), 3.86 (t, 2H), 3.7–3.6 (m, 8H), 3.56 (m, 2H), 3.45 (m, 2H), 3.06 (t, 2H), 2.33 (s, 6H) and 2.22 (m, 2H), 1.98 (s, 3H). *m/z* (ES) 511.2834 $(M + H)^+$. Calc. for $C_{29}H_{39}N_2O_6$: 511.2808.

Compound **22**: δ_H 7.6–7.2 (m, 7H), 6.4 (NH), 6.14 (s, 1H), 4.61 (s, 2H), 3.86 (t, 2H), 3.7–3.5 (m, 14H), 3.44 (m, 2H), 3.06 (t, 2H), 2.33 (s, 6H), 2.22 (m, 2H), 1.97 (s, 3H). *m/z* (ES) 577.2886 $(M + Na)^+$. Calc. for $C_{31}H_{42}N_2NaO_7$: 577.2890.

Compound **24**: δ_H 7.6–7.2 (m, 7H), 6.4 (NH), 6.14 (s, 1H), 4.61 (s, 2H), 3.86 (t, 2H), 3.7–3.5 (m, 22H), 3.44 (m, 2H), 3.06 (t, 2H), 2.33 (s, 6H), 2.22 (m, 2H), 1.98 (s, 3H). *m/z* (ES) 665.3384 $(M + Na)^+$. Calc. for $C_{35}H_{50}N_2NaO_9$: 665.3414.

*1,8-Bis[5-{3-(2,6-dimethyl-4-phenylphenoxy)propyl}isoxazolyl-3-methyloxy]-3,6-dioxaoctane **25***

Sodium hydride (60% in oil, 4 mg, 93 μmol) was added to a solution of triethylene glycol compound **11** (35 mg, 75 μmol) in THF (2 mL) and then, after stirring the reaction mixture under an atmosphere of argon for 1 h, tetrabutylammonium iodide (10 mg) and a solution of the bromomethyl compound **8** (30 mg, 75 μmol) in THF (1.5 mL) were added and the mixture was allowed to stir overnight. After addition of saturated ammonium chloride (1 mL), the reaction mixture was partitioned between ethyl acetate (50 mL) and water (15 mL). The organic phase was washed with brine, dried (Na_2SO_4), and then concentrated to give a pale yellow oil. The crude product was chromatographed on silica gel (10 g), using as eluent 1 : 1 ethyl acetate/hexanes, to give compound **25** (21 mg, 35%) as a colourless oil. δ_H 7.6–7.2 (m, 14H), 6.14 (s, 2H), 4.61 (s, 4H), 3.86 (t, 4H), 3.67 (s, 12H), 3.06 (t, 4H), 2.32 (s, 12H), 2.22 (m, 4H). *m/z* (ES) 811.3947 $(M + Na)^+$. Calc. for $C_{48}H_{56}N_2NaO_8$: 811.3934.

*General Procedure for the Preparation of Bis[5-{3-(2,6-dimethyl-4-phenylphenoxy)propyl}isoxazole-3-methyloxy]-PEG derivatives **26**–**30***

Compounds **26**–**30** were prepared by reaction of compounds **12**–**16** and the bromomethyl compound **8** using essentially the same method as that described above for compound **25**. The compounds were purified on silica gel, and characterized by their NMR spectra and mass spectrometric data.

Compound **26**: δ_H 7.6–7.2 (m, 14H), 6.14 (s, 2H), 4.61 (s, 4H), 3.86 (t, 4H), 3.65 (s, 16H), 3.06 (t, 4H), 2.32 (s, 12H), and 2.22 (m, 4H). *m/z* (ES) 855.4160 $(M + Na)^+$. Calc. for $C_{50}H_{60}N_2NaO_9$: 855.4197.

Compound **27**: δ_H 7.6–7.2 (m, 14H), 6.14 (s, 2H), 4.61 (s, 4H), 3.86 (t, 4H), 3.65 (m, 24H), 3.06 (t, 4H), 2.32 (s, 12H) 2.22 (m, 4H). *m/z* (ES) 943.4737 $(M + Na)^+$. Calc. for $C_{54}H_{68}N_2NaO_{11}$: 943.4721.

Compound **28**: δ_H 7.6–7.2 (m, 14H), 6.14 (s, 2H), 4.61 (s, 4H), 3.86 (t, 4H), 3.7–3.6 (m, 29H), 3.06 (t, 4H), 2.32 (s, 12H), 2.22 (m, 4H). *m/z* (ES) 1075.5515 $(M + Na)^+$. Calc. for $C_{60}H_{80}N_2NaO_{14}$: 1075.5507.

Compound **29**: δ_H 7.6–7.2 (m, 14H), 6.14 (s, 2H), 4.61 (s, 4H), 3.86 (t, 4H), 3.7–3.6 (m, 50H), 3.06 (t, 4H), 2.32 (s, 12H), 2.22 (m, 4H). *m/z* (ES) 1251.6609 $(M + Na)^+$. Calc. for $C_{68}H_{96}N_2NaO_{18}$: 1251.6556.

Compound **30**: δ_H 7.6–7.2 (m, 14H), 6.14 (s, 2H), 4.61 (s, 4H), 3.87 (t, 4H), 3.7–3.6 (m, 85H), 3.06 (t, 4H), 2.32 (s, 12H), 2.22 (m, 4H). *m/z* (ES) 1647.9894 $(M + Na)^+$. Calc. for $C_{86}H_{132}N_2NaO_{27}$: 1647.8915.

*1,4-Bis([5-{3-(2,6-dimethyl-4-phenylphenoxy)propyl}isoxazolyl-3-methyloxy]ethoxyethoxymethyl)benzene **31***

Sodium hydride (60% in oil, 5 mg, 123 μmol) was added to a solution of compound **10** (35 mg, 82 μmol) in THF (2 mL), then, after 1 h of stirring the mixture under argon, tetrabutylammonium iodide (10 mg) and α,α′-dibromo-*p*-xylene (10.5 mg, 41 μmol) were added and the reaction mixture was allowed to stir overnight. The reaction mixture was quenched with saturated ammonium chloride and then partitioned between ethyl acetate (50 mL) and water (10 mL). The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. Chromatography of the crude residue on silica gel (12 g), eluent 98.5 : 1.5 dichloromethane/methanol, gave the *title compound* **31** (19 mg, 48%) as a colourless oil. δ_H 7.6–7.2 (m, 18H), 6.14 (s, 2H), 4.62 (s, 4H), 4.55 (s, 4H), 3.85 (t, 4H), 3.7–3.55 (m, 16H), 3.05 (t, 4H), 2.32 (s, 12H), 2.21 (m, 4H). *m/z* (ES) 975.4748 $(M + Na)^+$. Calc. for $C_{58}H_{68}N_2NaO_{10}$: 975.4772.

*General Procedure for the Preparation of Bis[5-{3-(2,6-dimethyl-4-phenylphenoxy)propyl}isoxazole-3-methyloxy-PEG]-*p*-xylene Derivatives **32**–**34***

Compounds **32**–**34** were prepared by reaction of compounds **11**–**13** and α, α′-dibromo-*p*-xylene using essentially the same method as described above for compound **31**. The compounds were purified on silica gel, and characterized by their NMR spectra and mass spectrometric data.

Compound **32**: δ_H 7.6–7.2 (m, 18H), 6.14 (s, 2H), 4.61 (s, 4H), 4.54 (s, 4H), 3.85 (t, 4H), 3.7–3.55 (m, 24H), 3.05 (t, 4H), 2.32 (s, 12H), 2.22 (m, 4H). *m/z* (ES) 1063.5182 $(M + Na)^+$. Calc. for $C_{62}H_{76}N_2NaO_{12}$: 1063.5296.

Compound **33**: δ_H 7.6–7.2 (m, 18H), 6.14 (s, 2H), 4.61 (s, 4H), 4.54 (s, 4H), 3.86 (t, 4H), 3.7–3.55 (m, 32H), 3.06 (t, 4H), 2.32 (s, 12H), 2.22 (m, 4H). *m/z* (ES) 1151.5792 $(M + Na)^+$. Calc. for $C_{66}H_{84}N_2NaO_{14}$: 1151.5820.

Compound **34**: δ_H 7.6–7.2 (m, 18H), 6.14 (s, 2H), 4.61 (s, 4H), 4.54 (s, 4H), 3.86 (t, 4H), 3.7–3.55 (m, 48H), 3.06 (t, 4H), 2.32 (s, 12H), 2.22 (m, 4H). *m/z* (ES) 1327.7009 $(M + Na)^+$. Calc. for $C_{74}H_{100}N_2NaO_{18}$: 1327.6869.

*1,3-Bis[5-{3-(2,6-dimethyl-4-phenylphenoxy)propyl}-3-isoxazolylmethoxy(ethoxyethylureido)]-4-methylbenzene **35***

Toluene-2,4-diisocyanate (8 mg, 46 μmol) was added to a solution of aminoethoxy compound **17** (43 mg, 101 μmol) in DMF (1.5 mL) containing triethylamine (10 mg, 101 μmol), and then the reaction mixture

was allowed to stir under an atmosphere of argon for 4 days. The reaction was adsorbed onto silica gel (1 g) and chromatographed on silica gel (10 g), eluent 96 : 4 dichloromethane/methanol, to give the *title compound* **35** (38 mg, 73%) as a colourless oil. δ_H 7.6–7.2 (m, 17H), 6.12 (s, 1H), 6.10 (s, 1H), 4.63 (s, 2H), 4.59 (s, 2H), 3.85 (m, 4H), 3.7–3.5 (m, 12H), 3.42 (m, 4H), 3.05 (m, 4H), 2.31 (s, 12H), 2.21 (m, 4H), 2.13 (s, 3H). *m/z* (ES) 1045.5100 $(M + Na)^+$. Calc. for $C_{59}H_{70}N_6NaO_{10}$: 1045.5051.

*General Procedure for the Preparation of 1,3-Bis[5-{3-(2,6-dimethyl-4-phenylphenoxy)propyl}-3-isoxazolylmethoxy(PEG-ethylureido)]-4-methylbenzene Derivatives **36**–**38***

Compounds **36**–**38** were prepared by reaction of compounds **19**, **21**, and **23** with toluene-2,4-diisocyanate using essentially the same method as that described above for compound **35**. The compounds were purified on silica gel, and characterized by their NMR and mass spectrometric data.

Compound **36**: δ_H 7.6–7.2 (m, 17H), 6.14 (s, 1H), 6.08 (s, 1H), 4.64 (s, 2H), 4.60 (s, 2H), 3.84 (m, 4H), 3.7–3.5 (m, 20H), 3.40 (m, 4H), 3.03 (m, 4H), 2.31 (s, 12H), 2.18 (m, 4H), 2.11 (s, 3H). *m/z* (ES) 1133.5605 $(M + Na)^+$. Calc. for $C_{63}H_{78}N_6NaO_{12}$: 1133.5575.

Compound **37**: δ_H 7.6–7.2 (m, 17H), 6.08 (s, 2H), 4.56 (s, 2H), 4.55 (s, 2H), 3.84 (t, 4H), 3.7–3.5 (m, 28H), 3.40 (m, 4H), 3.02 (m, 4H), 2.32 (s, 12H), 2.18 (m, 4H), 2.14 (s, 3H). *m/z* (ES) 1221.6117 $(M + Na)^+$. Calc. for $C_{67}H_{86}N_6NaO_{14}$: 1221.6100.

Compound **38**: δ_H 7.6–7.0 (m, 17H), 6.12 (s, 2H), 4.57 (s, 4H), 3.85 (m, 4H), 3.7–3.5 (m, 44H), 3.41 (m, 4H), 3.05 (m, 4H), 2.32 (s, 12H), 2.21 (m, 4H), 2.16 (s, 3H). *m/z* (ES) 1397.7187 $(M + Na)^+$. Calc. for $C_{75}H_{102}N_6NaO_{18}$: 1397.7148.

*3-(t-Butyldimethylsilyloxymethyl)-5-(3-t-butyldiphenylsilyloxypropyl)isoxazole **39***

t-Butyldiphenylsilyl chloride (6.0 g, 22 mmol) was added to a solution of 3-(*t*-butyldimethylsilyloxymethyl)-5-(3-hydroxypropyl)isoxazole **6** (4.74 g, 17.5 mmol) and imidazole (1.55 g, 22.7 mmol) in anhydrous DMF (5 mL), and then the reaction mixture was stirred overnight under argon. The reaction mixture was concentrated under reduced pressure, and then taken up in hexane (300 mL), washed with water (3 × 50 mL), and brine. The organic phase was dried (Na_2SO_4) and concentrated before being chromatographed on silica gel (300 g), eluent 97 : 3 hexane/ethyl acetate, to give the *title compound* **39** (8.3 g, 93%). δ_H 7.66 (m, 4H), 7.42 (m, 6H), 6.00 (s, 1H), 4.72 (s, 2H), 3.71 (t, 2H), 2.88 (t, 2H), 1.94 (m, 2H), 1.06 (s, 9H), 0.92 (s, 9H), 0.10 (s, 6H). *m/z* (ES) 510.2887 $(M + H)^+$. Calc. for $C_{29}H_{44}NO_3Si_2$: 510.2860.

*5-(3-t-Butyldiphenylsilyloxypropyl)-3-hydroxymethylisoxazole **40***

The *t*-butyldimethylsilyloxy group in compound **39** was hydrolyzed under acidic conditions (THF/1 M HCl) to give the *title compound* **40** as a colourless oil in 91% yield. The ^1H NMR spectrum was consistent with the published data.[20] *m/z* (ES) 396.2009 $(M + H)^+$. Calc. for $C_{23}H_{30}NO_3Si$: 396.1995.

*3-(Bromomethyl)-5-(3-t-butyldiphenylsilyloxypropyl)isoxazole **41***

Bromination of compound **40** with triphenylphosphine/bromine following the published method[22] gave the *bromomethyl* compound **41** in 75% yield. δ_H 7.66 (m, 4H), 7.42 (m, 6H), 6.00 (s, 1H), 4.37 (s, 2H), 3.71 (t, 2H), 2.89 (t, 2H), 1.94 (m, 2H), 1.07 (s, 9H). *m/z* (ES) 480.0959 $(M + Na)^+$. Calc. for $C_{23}H_{28}BrNNaO_2Si$: 480.0970.

*5-(3-t-Butyldiphenylsilyloxypropyl)-3-(2-hydroxyethoxy)-ethoxymethylisoxazole **42***

Reaction of the bromomethyl compound **41** with diethylene glycol and sodium hydride in tetrahydrofuran using essentially the same method as described for compound **10** gave the *title compound* as an oil (67%). δ_H 7.65 (m, 4H), 7.39 (m, 6H), 6.02 (s, 1H), 4.60 (s, 2H), 3.67 (m, 10H), 2.89 (t, 2H), 1.94 (m, 2H), 1.06 (s, 9H). *m/z* (ES) 506.2343 $(M + Na)^+$. Calc. for $C_{27}H_{37}NNaO_5Si$: 506.2339.

*1,5-Bis{5-[3-(t-butyldiphenylsilyloxypropyl)]isoxazolyl-3-methyloxy}-3-oxapentane **43***

Sodium hydride (60% in oil, 16 mg, 0.39 mmol) was added to a solution of compound **42** (127 mg, 0.26 mmol), tetrabutylammonium iodide (10 mg), and compound **41** (120 mg, 0.26 mmol) in THF, and then the reaction mixture was allowed to stir overnight under argon. The reaction was quenched with saturated ammonium chloride, and then the mixture was partitioned between ethyl acetate (3 × 25 mL) and brine (10 mL). The organic phase was dried (Na_2SO_4) and concentrated. Chromatography of the crude residue on silica gel (20 g), eluent 75 : 25 hexane/ethyl acetate, gave the *title compound* **43** as a colourless oil (144 mg, 64%). δ_H 7.62 (m, 8H), 7.40 (m, 12H), 6.00 (s, 2H), 4.58 (s, 4H), 3.70 (t, 4H), 3.65 (s, 8H), 2.87 (t, 4H), 1.92 (m, 4H), 1.05 (s, 18H).

*1,5-Bis[5-[3-hydroxypropyl]isoxazolyl-3-methyloxy]-3-oxapentane **44***

A solution of tetrabutylammonium fluoride (1 M, 0.465 mL, 0.465 mmol) in THF was added to a solution of the adduct **43** (133 mg, 0.155 mmol) in THF (3 mL) and the reaction mixture was stirred overnight under argon. The mixture was concentrated, and then partitioned between brine (5 mL) and ethyl acetate (3 × 20 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. Chromatography of the crude residue on silica gel (7.5 g), eluent 96 : 4 CH_2Cl_2/methanol, gave the *title compound* **44** as a colourless oil (57 mg, 96%). δ_H 6.09 (s, 2H), 4.57 (s, 4H), 3.67 (t, 4H), 3.64 (s, 8H), 2.84 (t, 4H), 1.92 (m, 4H).

*1,5-Bis[5-[3-[2,6-dimethyl-4-(5-trifluoromethyl-1,2,4-oxadiazolyl)-phenoxy]propyl]isoxazolyl-3-methyloxy]-3-oxapentane **46***

Diisopropylazodicarboxylate (38 mg, 189 μmol) was added to an ice-cold solution of the bis(hydroxypropyl) compound **44** (29 mg, 76 μmol), triphenylphosphine (50 mg, 189 μmol), and 2,6-dimethyl-4-(5-trifluoromethyl-1,2,4-oxadiazolyl)phenol[25] **45** (49 mg,189 μmol) in ether (1 mL), and then the reaction mixture was allowed to warm to room temperature and stirred overnight under argon. The reaction mixture was filtered, concentrated, and then the crude residue was chromatographed on silica gel (10 g), eluent 2 : 1 hexane/ethyl acetate, to give the *dimeric compound* **46** as a colourless oil (48 mg, 73%). δ_H [$(CD_3)_2CO$] 7.77 (s, 4H), 6.31 (s, 2H), 4.58 (s, 4H), 3.97 (t, 4H), 3.64 (s, 8H), 3.09 (t, 4H), 2.36 (s, 12H), 2.25 (m, 4H). δ_F [$(CD_3)_2CO$] 65.5.

*General Procedure for the Preparation of Bis[5-{3-(2,6-dimethyl-4-[5-trifluoromethyl-1,2,4-oxadiazolyl]phenoxy)propyl}isoxazolyl-3-methyloxy]-PEG derivatives **47**–**49***

Compounds **47**–**49** were prepared using essentially the same method as described above for compound **46** by using the appropriate glycols. The compounds were purified on silica gel, isolated as colourless oils, and characterized by their NMR spectra and mass spectrometric data.

Compound **47**: δ_H [$(CD_3)_2CO$] 7.82 (s, 4H), 6.34 (s, 2H), 4.61 (s, 4H), 4.02 (t, 4H), 3.69 (s, 8H), 3.64 (s, 4H), 3.13 (t, 4H), 2.41 (s, 12H), 2.30 (m, 4H). δ_F [$(CD_3)_2CO$] 65.46. *m/z* (ES) 931.3059 $(M + Na)^+$. Calc. for $C_{42}H_{46}F_6N_6NaO_{10}$: 931.3077.

Compound **48**: δ_H [$(CD_3)_2CO$] 7.78 (s, 4H), 6.30 (s, 2H), 4.56 (s, 4H), 3.97 (t, 4H), 3.7–3.5 (m, 16H), 3.09 (t, 4H), 2.37 (s, 12H), 2.26 (m, 4H). δ_F [$(CD_3)_2CO$] 65.28. *m/z* (ES) 975.3288 $(M + Na)^+$. Calc. for $C_{44}H_{50}F_6N_6NaO_{11}$: 975.3339.

Compound **49**: δ_H [$(CD_3)_2CO$] 7.78 (s, 4H), 6.31 (s, 2H), 4.57 (s, 4H), 3.98 (t, 4H), 3.7–3.5 (m, 24H), 3.09 (t, 4H), 2.37 (s, 12H), 2.26 (m, 4H). δ_F [$(CD_3)_2CO$] 65.31. *m/z* (ES) 1063.3819 $(M + Na)^+$. Calc. for $C_{48}H_{58}F_6N_6NaO_{13}$: 1063.3864.

*1,4-Bis([5-{3-(t-butyldiphenylsilyloxypropyl)}isoxazolyl-3-methyloxy]ethoxyethoxymethyl)benzene **50***

Sodium hydride (60% in paraffin oil, 21 mg, 0.52 mmol) was added to a solution of compound **42** (169 mg, 0.35 mmol), α,α′-dibromo-*p*-xylene (44 mg, 0.17 mmol), and tetrabutylammonium iodide (13 mg) in

THF, and the reaction mixture was left to stir overnight under argon. After addition of saturated ammonium chloride (1 mL), the mixture was partitioned between brine (10 mL) and ethyl acetate (2 × 50 mL). The combined organic phases were dried (Na_2SO_4) and then concentrated under reduced pressure. Chromatography of the crude residue on silica gel (2 × 15 g), eluents 3 : 2 hexane/ethyl acetate and then 98.5 : 1.5 dichloromethane/methanol, gave the *title compound* as a colourless oil (86 mg, 46%). δ_H 7.65 (m, 8H), 7.40 (m, 12H), 7.30 (s, 4H), 6.01 (s, 2H), 4.59 (s, 4H), 4.54 (s, 4H), 3.70 (t, 4H), 3.7–3.55 (m, 16H), 2.87 (t, 4H), 1.92 (m, 4H), 1.05 (s, 18H). *m/z* (ES) 1091.5187 $(M + Na)^+$. Calc. for $C_{62}H_{80}N_2NaO_{10}Si_2$: 1091.5249.

1,4-Bis([5-{3-[2,6-dimethyl-4-(5-trifluoromethyl-1,2,4-oxadiazolyl)-phenoxy]propyl}isoxazolyl-3-methyloxy]ethoxyethoxymethyl)-benzene ***51***

A solution of tetrabutylammonium fluoride (1 M, 225 μL, 225 μmol) in THF was added to a solution of compound **50** (80 mg, 75 μmol) in THF (3 mL). After being stirred overnight under argon, the reaction mixture was concentrated and the residue chromatographed on silica gel (7.5 g), eluent 96 : 4 dichloromethane/methanol, to give the bis(hydroxyl) compound *1,4-bis([5-{3-(hydroxypropyl)}isoxazolyl-3-methyloxy]ethoxyethoxymethyl)benzene* (40 mg, 90%). δ_H 7.30 (s, 4H), 6.09 (s, 2H), 4.59 (s, 4H), 4.54 (s, 4H), 3.60 (m, 20H), 2.82 (t, 4H), 1.90 (m, 4H). *m/z* (ES) 615.2920 $(M + Na)^+$. Calc. for $C_{30}H_{44}N_2NaO_{10}$: 615.2894. Reaction of this bridging compound with two equivalents of 2,6-dimethyl-4-(5-trifluoromethyl-1,2,4-oxadiazolyl)phenol **45** using essentially the same method as described for compound **46** gave the *title compound* **51** (32 mg, 50%). δ_H [$(CD_3)_2CO$] 7.82 (s, 4H), 7.36 (s, 4H), 6.32 (s, 2H), 4.62 (s, 4H), 4.57 (s, 4H), 4.01 (s, 4H), 3.75–3.6 (m, 16H), 3.11 (t, 4H), 2.41 (s, 12H), 2.29 (m, 4H). δ_F [$(CD_3)_2CO$] 65.5. *m/z* (ES) 1095.3885 $(M + Na)^+$. Calc. for $C_{52}H_{58}F_6N_6NaO_{12}$: 1095.3915.

General Procedure for the Preparation of 1,4-Bis[5-{3-[2,6-dimethyl-4-(5-trifluoromethyl-1,2,4-oxadiazolyl)phenoxy]propyl}-isoxazole-3-methyloxy-PEG]-p-*xylene Derivatives* ***52–54***

Compounds **52**–**54** were prepared using essentially the same method as described above for compound **51** by starting with compound **41** and the appropriate polyethylene glycol. The compounds were purified on silica gel, isolated as colourless oils, and characterized by their NMR spectra and mass spectrometric data as outlined below.

Compound **52**: δ_H [$(CD_3)_2CO$] 7.78 (s, 4H), 7.31 (s, 4H), 6.30 (s, 2H), 4.56 (s, 4H), 4.52 (s, 4H), 3.96 (t, 4H), 3.75–3.6 (m, 24H), 3.08 (t, 4H), 2.37 (s, 12H), 2.25 (m, 4H). δ_F [$(CD_3)_2CO$] 65.46. *m/z* (ES) 1183.4427 $(M + Na)^+$. Calc. for $C_{56}H_{66}F_6N_6NaO_{14}$: 1183.4439.

Compound **53**: δ_H [$(CD_3)_2CO$] 7.83 (s, 4H), 7.46 (s, 4H), 6.34 (s, 2H), 4.60 (s, 4H), 4.56 (s, 4H), 4.01 (t, 4H), 3.75–3.6 (m, 32H), 3.13 (t, 4H), 2.41 (s, 12H), 2.09 (m, 4H). δ_F [$(CD_3)_2CO$] 65.27. *m/z* (ES) 1271.5009 $(M + Na)^+$: Calc. for $C_{60}H_{74}F_6N_6NaO_{16}$: 1271.4963.

Compound **54**: δ_H [$(CD_3)_2CO$] 7.84 (s, 4H), 7.37 (s, 4H), 6.34 (s, 2H), 4.61 (s, 4H), 4.58 (s, 4H), 4.03 (t, 4H), 3.7–3.6 (m, 48H), 3.13 (t, 4H), 2.42 (s, 12H), 2.3 (m, 4H). δ_F [$(CD_3)_2CO$] 65.44. *m/z* (ES) 1447.5934 $(M + Na)^+$. Calc. for $C_{68}H_{90}F_6N_6NaO_{20}$: 1447.6012.

Bis(4-{2-[1-(6-chloro-3-pyridazinyl)-4-piperidinyl]ethoxy}-benzaldehyde Oxime)tetraethylene Glycol Ether ***59***

1,11-Diaminooxy-3,6,9-trioxaundecane **58** was prepared by hydrolysis of the bisether formed from tetraethylene glycol and *N*-hydroxyphthalimide as previously reported,[29] except that 6 M hydrochloric acid was used instead of hydrazine. The *bis(alkoxyamine)* was isolated as the bis(hydrochloride) salt, δ_H (CD_3OD) 4.2 (m, 4H), 3.8 (m, 4H), 3.65 (s, 8H). A solution of 4-{2-[1-(6-chloro-3-pyridazinyl)-4-piperidinyl]ethoxy}benzaldehyde[26] **56** (125 mg, 0.4 mmol) and the bis(hydrochloride) of compound **58** (50 mg, 0.17 mmol) in ethanol (20 mL) was mixed with a solution of sodium hydroxide (40 mg, 1 mmol) in water (0.4 mL), and the solution was stirred at room temperature for 72 h. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was purified by chromatography on silica gel (30 g) with chloroform as eluent. The *title compound* **59** was isolated as a thick, pale yellow oil (50 mg, 24%). δ_H 8.04 (s, 2H), 7.48 (d, 4H), 7.17 (d, 2H), 6.90 (d, 4H), 6.83 (d, 2H), 4.4–4.25 (m, 8H), 4.02 (t, 4H), 3.76 (m, 4H), 3.67 (s, 8H), 2.92 (t, 4H), 1.95–1.7 (m, 10H), 1.4–1.2 (m, 4H). *m/z* (ES) 881.9 (70%), 879.9 (100, M + H), 768 (12), 440.8 (30).

Measurement of Anti-HRV Activity in Mammalian Cell Culture Assays: Inhibition of Viral Cytopathic Effect (CPE) and Measurement of Cytotoxicity

The ability of compounds to suppress virus replication and thereby protect cells from HRV-induced CPE was measured in two cell lines: (1) human Negroid cervix epithelial HeLa (Ohio) for HRV-2; and (2) human embryo lung (MRC-5) for HRV-1A.

Cells were grown in 96-well tissue culture plates using conventional mammalian tissue culture medium supplemented with foetal calf serum. The antiviral potency of the test compound was assessed by exposing replicate tissue culture wells to a selected dilution series of between six and seven compound concentrations in the presence of the minimum test virus inoculum sufficient to invoke significant CPE over the course of the assay. Control cells were also exposed to identical concentrations of compounds in the absence of virus or were infected with virus under the same conditions but in the absence of compound. Control compounds of established anti-HRV efficacy with known capsid-binding properties (pleconaril and pirodavir) were assayed in parallel to test compounds. The assays were incubated at 33°C in a 5% CO_2 atmosphere until significant CPE was observed (on average 5 days but in some cases 8 days), at which time cell viaibility was quantified by vital dye metabolism (MTT and XTT) and/or vital dye uptake (Neutral Red). Dye metabolism/uptake was quantified spectrophotometrically. The 50% cytotoxicity concentration (CC_{50}) was defined as the concentration of the test compound that reduced the absorbance of the mock infected cells by 50% of control value. The 50% effective concentration (EC_{50}) was defined as the concentration which protected 50% of the cells from virus cytopathology. Both were determined graphically.

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References

[1] M. J. Makela, T. Puhakka, O. Ruuskanen, M. Leinonen, P. Saikku, M. Kimpimaki, S. Blomqvist, T. Hyypia, et al., *J. Clin. Microbiol.* **1998**, *36*, 539.

[2] R. B. Turner, *Pediatr. Ann.* **1998**, *27*, 790.

[3] M. A. McKinlay, *Curr. Opin. Pharmacol.* **2001**, *1*, 477. doi:10.1016/S1471-4892(01)00083-2

[4] G. D. Diana, D. C. Pevear, *Antivir. Chem. Chemother.* **1997**, *8*, 401.

[5] M. J. Tebbe, W. A. Spitzer, F. Victor, S. C. Miller, C. C. Lee, T. R. Sattelberg, E. McKinney, J. C. Tang, *J. Med. Chem.* **1997**, *40*, 3937. doi:10.1021/JM970423K

[6] P. S. Dragovich, T. J. Prins, R. Zhou, S. E. Webber, J. T. Marakovits, S. A. Fuhrman, A. K. Patick, D. A. Matthews, et al., *J. Med. Chem.* **1999**, *42*, 1213. doi:10.1021/JM9805384

[7] F. G. Hayden, D. T. Herrington, T. L. Coats, K. Kim, E. C. Cooper, S. A. Villano, S. Liu, S. Hudson, et al., *Clin. Infect. Dis.* **2003**, *36*, 1523. doi:10.1086/375069

[8] For selected examples of X-ray structures of HRV with bound capsid binders see:
(*a*) K. H. Kim, P. Willingmann, Z. X. Gong, M. J. Kremer, M. S. Chapman, I. Minor, M. A. Oliviera, M. G. Rossmann, et al., *J. Mol. Biol.* **1993**, *230*, 206. doi:10.1006/JMBI.1993.1137

(*b*) D. A. Oren, A. Zhang, H. Nesvadba, B. Rosenwirth, E. Arnold, *J. Mol. Biol.* **1996**, *259*, 120. doi:10.1006/JMBI.1996.0307
(*c*) V. L. Giranda, G. R. Russo, P. J. Felock, T. R. Bailey, T. Draper, D. J. Aldous, J. Guiles, F. J. Dutko, et al., *Acta Crystallogr.* **1995**, *D51*, 496.

[9] G. D. Diana, P. Kowalczyk, A. M. Treasurywala, R. C. Oglesby, D. C. Pevear, F. J. Dutko, *J. Med. Chem.* **1992**, *35*, 1002.

[10] (*a*) M. Mammen, S.-K. Choi, G. M. Whitesides, *Angew. Chem. Int. Ed.* **1998**, *37*, 2754. doi:10.1002/(SICI)1521-3773(19981102)37:20<2754::AID-ANIE2754>3.3.CO;2-V
(*b*) S. Borman, *Chem. Eng. News* **2000**, *78(41)*, 48.

[11] D. Wright, L. Usher, *Curr. Org. Chem.* **2001**, *5*, 1107.

[12] J. E. Gestwicki, C. W. Cairo, L. E. Strong, K. A. Oetjen, L. L. Kiessling, *J. Am. Chem. Soc.* **2002**, *124*, 14922. doi:10.1021/JA027184X

[13] J. H. Griffin, M. S. Linsell, M. B. Nodwell, Q. Chen, J. L. Pace, K. L. Quast, K. M. Krause, L. Farrington, et al., *J. Am. Chem. Soc.* **2003**, *125*, 6517. doi:10.1021/JA021273S

[14] See for example: N. Schaschke, A. Dominik, G. Matschiner, C. P. Sommerhoff, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 985. doi:10.1016/S0960-894X(02)00063-X

[15] G. D. Glick, J. R. Knowles, *J. Am. Chem. Soc.* **1991**, *113*, 4701.

[16] G. B. Sigal, M. Mammen, G. Dahmann, G. M. Whitesides, *J. Am. Chem. Soc.* **1996**, *118*, 3789. doi:10.1021/JA953729U

[17] J. J. Landers, Z. Cao, I. Lee, L. T. Piehler, P. P. Myc, A. Myc, T. Hamouda, A. T. Galecki, et al., *J. Infect. Dis.* **2002**, *186*, 1222. doi:10.1086/344316

[18] K. G. Watson, R. Cameron, R. J. Fenton, D. Gower, S. Hamilton, B. Jin, G. Y. Krippner, A. Luttick, et al., *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1589. doi:10.1016/J.BMCL.2003.09.102

[19] Aspects of this work are the subject of *International Patent Application PCT WO 01/19822* **2001**.

[20] G. D. Diana, D. L. Volkots, T. J. Nitz, T. R. Bailey, M. A. Long, N. Vescio, S. Aldous, D. C. Pevear, F. J. Dutko, *J. Med. Chem.* **1994**, *37*, 2421.

[21] A. T. Hadfield, G. D. Diana, M. G. Rossmann, *Proc. Natl Acad. Sci. USA* **1999**, *96*, 14730. doi:10.1073/PNAS.96.26.14730

[22] G. D. Diana, D. Cutcliffe, D. L. Volkots, J. P. Mallamo, T. R. Bailey, N. Vescio, R. C. Oglesby, T. J. Nitz, et al., *J. Med. Chem.* **1993**, *36*, 3240.

[23] J. W. Guiles, G. D. Diana, D. C. Pevear, *J. Med. Chem.* **1995**, *38*, 2780.

[24] W. Watanabe, K. Konno, K. Ijichi, H. Inoue, T. Yokata, S. Shigeta, *J. Virol. Methods* **1994**, *48*, 257. doi:10.1016/0166-0934(94)90124-4

[25] G. D. Diana, P. Rudewicz, D. C. Pevear, T. J. Nitz, S. C. Aldous, D. J. Aldous, D. T. Robinson, T. Draper, et al., *J. Med. Chem.* **1995**, *38*, 1355.

[26] K. G. Watson, R. N. Brown, R. Cameron, D. K. Chalmers, S. Hamilton, B. Jin, G. Y. Krippner, A. Luttick, et al., *J. Med. Chem.* **2003**, *46*, 3181. doi:10.1021/JM0202876

[27] R. B. Greenwald, Y. H. Choe, C. D. Conover, K. Shum, D. Wu, M. Royzen, *J. Med. Chem.* **2000**, *43*, 475. doi:10.1021/JM990498J

[28] F. J. Dekker, N. J. de Mol, J. van Ameijde, M. J. E. Fischer, R. Ruijtenbeek, F. A. M. Redegeld, R. M. J. Liskamp, *ChemBioChem* **2002**, *3*, 238. doi:10.1002/1439-7633(20020301)3:2/3<238::AID-CBIC238>3.0.CO;2-W

[29] V. G. Shtamburg, A. A. Dmitrenko, A. P. Pleshkova, L. M. Pritykin, *Russ. J. Org. Chem.* **1993**, *29*, 1464.