Received: 3 May 2018 | Revised: 15 May 2018 | Accepted: 17 May 2018

DOI: 10.1002/chir.22985

WILEY

REGULAR ARTICLE

Evaluation of the Edman degradation product of vancomycin bonded to core-shell particles as a new HPLC chiral stationary phase

Garrett Hellinghausen[1] | Diego A. Lopez[2] | Jauh T. Lee[2] | Yadi Wang[1] | Choyce A. Weatherly[1] | Abiud E. Portillo[1] | Alain Berthod[3] | Daniel W. Armstrong[1,2]

[1]Department of Chemistry and Biochemistry, The University of Texas at Arlington, Arlington, Texas, USA

[2]AZYP, LLC, Arlington, Texas, USA

[3]Institute of Analytical Sciences CNRS, University of Lyon 1, Villeurbanne, France

Correspondence
Daniel W. Armstrong, The University of Texas at Arlington, Arlington, TX 76019, USA.
Email: sec4dwa@uta.edu

Funding information
French National Center for Scientific Research, Grant/Award Number: ISA-CNRS-UMR5280; Robert A. Welch Foundation, Grant/Award Number: Y0026

Abstract

A modified macrocyclic glycopeptide-based chiral stationary phase (CSP), prepared via Edman degradation of vancomycin, was evaluated as a chiral selector for the first time. Its applicability was compared with other macrocyclic glycopeptide-based CSPs: TeicoShell and VancoShell. In addition, another modified macrocyclic glycopeptide-based CSP, NicoShell, was further examined. Initial evaluation was focused on the complementary behavior with these glycopeptides. A screening procedure was used based on previous work for the enantiomeric separation of 50 chiral compounds including amino acids, pesticides, stimulants, and a variety of pharmaceuticals. Fast and efficient chiral separations resulted by using superficially porous (core-shell) particle supports. Overall, the vancomycin Edman degradation product (EDP) resembled TeicoShell with high enantioselectivity for acidic compounds in the polar ionic mode. The simultaneous enantiomeric separation of 5 racemic profens using liquid chromatography-mass spectrometry with EDP was performed in approximately 3 minutes. Other highlights include simultaneous liquid chromatography separations of rac-amphetamine and rac-methamphetamine with VancoShell, rac-pseudoephedrine and rac-ephedrine with NicoShell, and rac-dichlorprop and rac-haloxyfop with TeicoShell.

KEYWORDS

complementary behavior, Edman degradation, electrospray ionization liquid chromatography-mass spectrometry, enantiomer separations, superficially porous particles, teicoplanin, vancomycin

1 | INTRODUCTION

Macrocyclic glycopeptide antibiotics were first introduced as chiral selectors for liquid chromatography by Armstrong in the early 1990s.[1] These natural products are produced by bacterial fermentation. Purified and bonded to silica particles, they make useful chiral stationary phases (CSPs) with a broad spectrum of interactions and therefore applicability.[2] The macrocyclic glycopeptide-based CSPs are multimodal, meaning they are stable and efficient in normal phase (NP), reversed phase (RP), polar organic mode (POM), and polar ionic mode (PIM).[3,4] The most distinctive feature of macrocyclic glycopeptides as chiral selectors is their ionic character. All macrocyclic glycopeptides are ionizable, bearing primary or secondary amines rendering them positively charged at neutral and acidic pH values.[5-7] They also have a carboxylic acid bearing a negative charge at neutral and high pH values so

that the net charge is adjustable according to the mobile phase pH. This is the foundation of PIM, which utilizes 100% methanol containing trace amounts of acid and base or a nonvolatile salt to tune the charges on the chiral selector to effect ionizable enantiomers' retention and separation.[6] Many ionizable compounds can be separated in PIM, but sometimes it is beneficial to adjust the hydrogen bonding interactions by switching to POM, which contains a mixture of acetonitrile and methanol with acid and base.[3] Others favor RP, in which methanol is generally mixed with an ammonium salt with pH adjustment to enhance ionic interactions. In RP, a low pH is generally preferred for amines, while higher pH is favored for acids. All these modes are compatible with mass spectrometry, and usually NP is not necessary for enantiomeric separations with macrocyclic glycopeptides, while other CSPs depend on it. This is especially important to biological analysis, which depends on mass spectrometry sensitivity for thermally liable and complex samples.

Another feature of the macrocyclic glycopeptide class of chiral selectors is their complementary behavior.[5,8] If a separation of an enantiomeric pair is observed on a macrocyclic selector, say teicoplanin, chances are that a baseline separation of this pair will be observed on a different selector, say vancomycin. The large number of possible interactions and structural similarities between the different macrocyclic glycopeptides explain the observed complementary behavior, which provides an ease of method development. A plethora of native macrocyclic glycopeptides have been explored for their use as chiral selectors, not only in liquid chromatography (LC) but also capillary electrophoresis and super critical fluid chromatography.[1,4,5,7-19] Of these, vancomycin, teicoplanin, and ristocetin A were commercialized as the CHIROBIOTICs as well as teicoplanin's aglycone.[20] Since the recent development of superficially porous particles or core-shell particles, which offer high throughput and more effective separations, several studies have been explored using core-shell macrocyclic glycopeptide-based CSPs (TeicoShell [TS] and VancoShell [VS]).[21-25] This has been particularly useful to ultrafast chiral separations needed in second dimension (2D) LC.[24-29] However, many glycopeptides are costly and have limited availability, which has led to a need to understand the applicability and limitations of more available glycopeptides so further exploration can be made concerning useful modifications.

Comprehensive studies have indicated that vancomycin is most useful for the separation of basic amines, while teicoplanin is most useful for the separation of acids, specifically amino acids.[17,20,23] When exploring the structural interactions driving these separations, it is difficult to assess their separation mechanisms due to the diverse and complex interactions of each macrocyclic glycopeptide.[6] However, it is thought that the carboxylic acid located in the vancomycin structure might play an important role for the interaction with amines, while the primary amine in teicoplanin might be important to chiral recognition for acids.[6] Some studies have been done with modified macrocyclic glycopeptides, such as the crystalline degradation of vancomycin, which incorporates a second carboxylic acid moiety in the structure.[30-32] Recently, a modified macrocyclic glycopeptide-based CSP, NicoShell (NS), was used for the novel LC enantiomeric separation of nicotine from tobacco e-liquids and several nicotine-related compounds, including carcinogenic tobacco-specific nitrosamines.[21,22] NicoShell was further utilized for the separation of several chiral amines.[23] An effective methodology was proposed for core-shell CSPs and was used in this study to evaluate a new selector, the vancomycin Edman degradation product (EDP).[23] The EDP differs from native vancomycin by the loss the N-terminus leucine residue, leaving a primary amine[33] (Figure 1). A set of 50 biologically active chiral compounds including stimulants, nonsteroidal

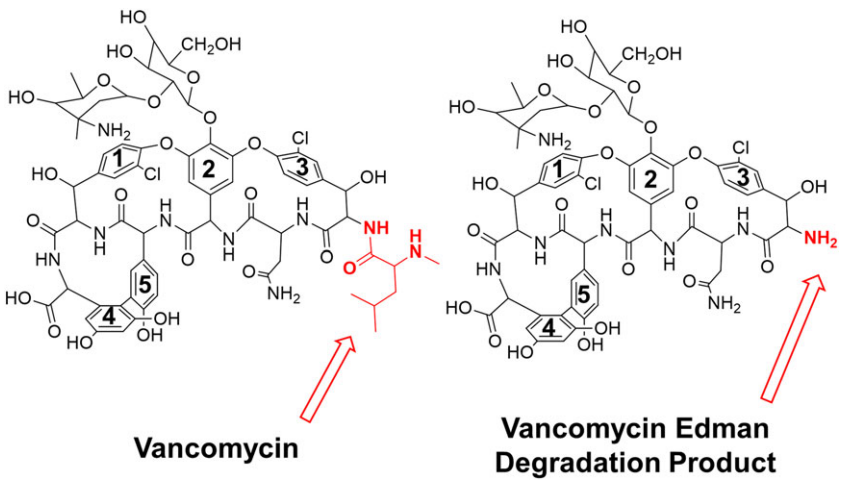


FIGURE 1 Structures of vancomycin and the vancomycin Edman degradation product. The five-aromatic ring association in the peptidic aglycone "basket" is labeled 1-5. The red arrow indicates the structural modification leading to the vancomycin Edman degradation product. See Section 2 for information concerning preparation of macrocyclic glycopeptide-based chiral stationary phases

anti-inflammatory drugs, pesticides, and a variety of acidic and basic pharmaceuticals were subjected to LC enantiomeric separation. Edman degradation product results were then compared with three other macrocyclic glycopeptide-based core-shell CSPs: TS, VS, and NS.

2 | MATERIALS AND METHODS

Macrocyclic glycopeptide-based core-shell CSPs (100 × 4.6 mm [i.d.]): vancomycin (VS), teicoplanin (TS), NS, and the vancomycin EDP were obtained from AZYP, LLC. (Arlington, Texas). The EDP selector was synthesized by reacting vancomycin with phenyl isothiocyanate in pyridine/water (50/50, v/v) followed by treatment with trifluoroacetic acid to selectively remove the N-terminal residue highlighted in red[33] in Figure 1. Thus, the hexapeptide derivative with a free primary amine (red arrow, Figure 1) was produced. The hexapeptide selector was then bonded to 2.7-μm core-shell particles, like the other CSPs, discussed in past literature, with a surface area of 120 m^2/g, a pore diameter of 12 nm, and a shell thickness of 0.5 μm.[1,25,29]

Analytes were purchased as racemic standards or individual enantiomer standards (then mixed to form racemates) from Cerilliant Corporation (Round Rock, Texas), Sigma-Aldrich (St Louis, Missouri), and LKT Laboratories Inc (Minneapolis, Minnesota). Racemic standards were prepared with methanol at 1 mg/mL for analysis. In the set of 50 selected analytes, 48 were ionizable compounds, mostly bases, since only 10 acidic compounds did not contain a nitrogen atom. Twenty-six analytes were amines or have an amine group in their structure. The remaining 14 nitrogen containing compounds were mostly amides (nine analytes) and a pyrrolizidine, pyran, benzoxazole, and two pyridine-containing compounds.

Solvents and additives including HPLC grade acetonitrile (ACN), methanol (MeOH), ethanol (EtOH), hexane (Hex), acetic acid (AA), trifluoroacetic acid (TFA), trimethylamine (TEA), formic acid (FA), ammonium formate (NH_4HCO_2), and ammonium trifluoroacetate (NH_4TFA) were obtained from Sigma-Aldrich (St Louis, Missouri). Water was purified by a Milli-Q water purification system (Millipore, Billerica, Massachusetts).

An Agilent 1260 (Agilent Technologies, Palo Alto, California) HPLC was used. It consisted of a 1200 diode array detector, autosampler, and quaternary pump. The mass spectrometer used in this study was a Shimadzu triple quadrupole liquid chromatography-mass spectrometry (LC-MS) instrument, LCMS-8040 (Shimadzu, Tokyo, Japan). All MS was operated in positive ion mode with an electron spray ionization source. The parameters were set as follows: nebulizer gas flow, 3 L/min; dryer gas flow, 15 L/min; desolvation line temperature, 250°C; heat block temperature, 400°C. Multiple ultraviolet (UV) wavelengths, 220, 230, and 254 nm, were utilized for detection and identification of enantiomers. All separations were carried out at room temperature, unless otherwise noted, using an isocratic method. Mobile phases were degassed by ultrasonication under vacuum for 5 minutes. Each analyte was screened in PIM, POM, RP, and NP. The screening mobile phase conditions referring to Table 1 were as follows: PIM: MeOH-NH_4Formate (100:0.1, v/w), POM: ACN-MeOH-AA-TEA (60:40:0.3:0.2, v/v/v/v), RP: MeOH-NH_4Formate (pH 3.6; 16 mM) (30:70, v/v), and NP: Hex-EtOH-TFA-TEA (70:30:0.3:0.2, v/v/v/v).

The dead time, t_0, was determined by the peak of the refractive index change due to the unretained sample solvent. Retention factors (k) were calculated using $k = (t_R - t_0)/(t_0)$, where t_R is the retention time of the first peak and t_0, the dead time of the column. Selectivity (α) was calculated using $\alpha = k_2/k_1$, where k_1 and k_2 are retention factors of the first and second peaks, respectively. Resolution (R_s) was calculated using the peak width at half peak height, $R_s = 2(t_{R2} - t_{R1})/(w_1 + w_2)$. Two EDP columns were produced and had a relative standard deviation (%RSD) within 5.0% for all R_s factors obtained.

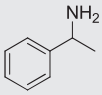
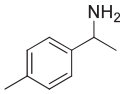
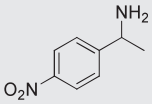
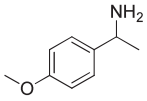
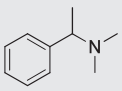
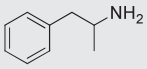
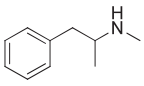
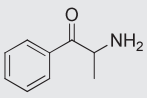
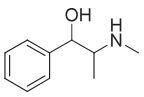
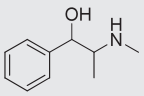
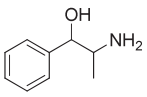
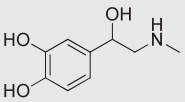
3 | RESULTS AND DISCUSSION

3.1 | Screening results

Preliminary screening with all four CSPs using 50 chiral compounds in each compatible chromatographic mode (PIM, POM, RP, and NP) was performed, making 200 analyses per CSP. When a partial separation of the enantiomers was obtained, this separation could be significantly improved by modulating the mobile phase as shown in previous studies, but it should be noted that this was not the aim of this study.[20,23] The best screening result (in terms of R_s) by each CSP are tabulated according to each compound in Table 1. In 46 cases (184 analyses), the compounds could not be separated on a CSP by all four mobile phases assayed. These are reported in Table 1 with $\alpha = 1.00$ and $R_s = 0.0$ in the Table 1. No k_1 was listed since four different values were obtained in the four modes tested, all four producing a single peak for the enantiomeric pair.

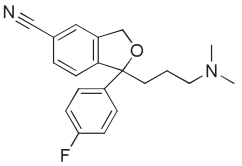
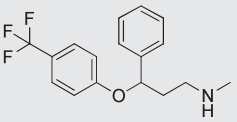
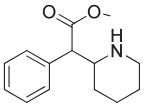
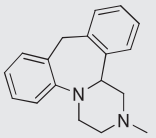
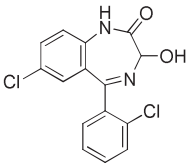
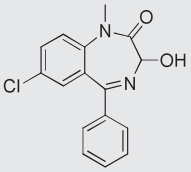
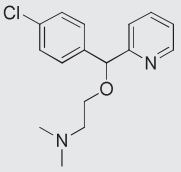
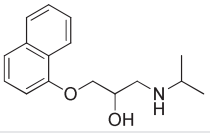
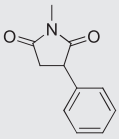
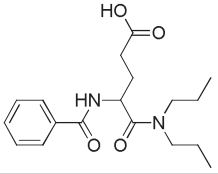
Overall, the screening procedure resulted in 40 racemic compounds (80%) baseline separated ($R_s \geq 1.5$) (Table 1). Several had $R_s \geq 1.5$ with more than one CSP; one compound (methylphenidate) separated on all four CSPs, five compounds on three of the CSPs, 17

TABLE 1 Chiral separation comparisons using core-shell macrocyclic glycopeptide-based CSPs

Name[a]	Structure[a]	CSP[b]	MP[c]	k_1[d]	α[d]	R_s[d]
(a) Chemical amines						
α-Methylbenzylamine		VS	PIM	0.9	1.07	0.8
		NS	POM	5.8	1.17	2.4
		EDP	PIM	0.4	1.05	0.3
		TS	...	...	1.00	0.0
α,4-Dimethylbenzylamine		VS	POM	3.0	1.12	1.4
		NS	POM	2.8	1.09	1.0
		EDP	POM	1.0	1.08	0.6
		TS	...	...	1.00	0.0
α-Methyl-4-nitrobenzylamine		VS	PIM	1.5	1.07	0.9
		NS	PIM	4.3	1.06	1.2
		EDP	PIM	0.6	1.04	0.3
		TS	NP	7.0	1.02	0.4
4-Methoxymethylbenzylamine		VS	PIM	1.0	1.45	5.1
		NS	PIM	2.2	1.08	1.6
		EDP	PIM	0.4	1.40	2.4
		TS	NP	4.6	1.03	0.5
N,N-α-Trimethylbenzylamine		VS	RP	0.3	1.23	1.3
		NS	PIM	2.7	1.11	1.6
		EDP	...	...	1.00	0.0
		TS	NP	5.5	1.03	0.6
(b) Stimulants						
Amphetamine		VS	PIM	1.0	1.17	1.7
		NS	...	...	1.00	0.0
		EDP	NP	2.0	1.12	1.6
		TS	...	...	1.00	0.0
Methamphetamine		VS	PIM	1.3	1.11	1.6
		NS	PIM	4.0	1.02	0.4
		EDP	NP	1.8	1.14	1.8
		TS	...	...	1.00	0.0
β-Ketoamphetamine (cathionine)		VS	PIM	0.9	1.18	1.6
		NS	PIM	2.3	1.80	8.3
		EDP	PIM	0.4	1.12	0.6
		TS	NP	5.4	1.11	1.1
(1 *RS*; 2 *SR*)-ephedrine		VS	POM	2.5	1.02	0.4
		NS	POM	6.2	1.13	2.0
		EDP	NP	1.9	1.01	0.2
		TS	POM	4.8	1.03	0.6
(1 *RS*; 2 *RS*)-pseudoephedrine		VS	POM	2.5	1.08	1.4
		NS	POM	4.7	1.38	5.0
		EDP	NP	2.5	1.12	1.6
		TS	POM	5.4	1.09	1.4
Norephedrine		VS	NP	3.2	1.03	0.4
		NS	PIM	2.4	1.07	1.3
		EDP	NP	2.3	1.04	0.6
		TS	PIM	2	1.02	0.4
Epinephrine		VS	...	...	1.00	0.0
		NS	POM	1.7	1.06	1.0
		EDP	...	...	1.00	0.0
		TS	POM	8.7	1.04	0.5

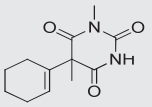
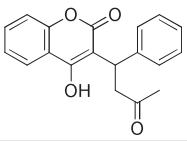
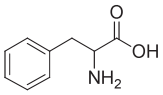
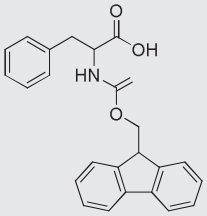
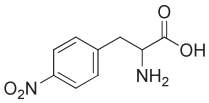
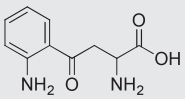
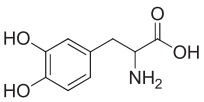
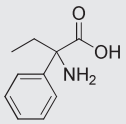
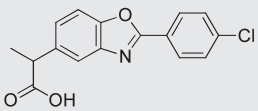
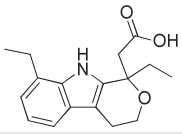
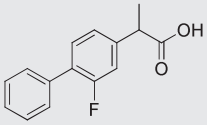
(Continues)

TABLE 1 (Continued)

Name[a]	Structure[a]	CSP[b]	MP[c]	k_1[d]	α[d]	R_s[d]
Citalopram		VS	PIM	1.8	1.13	1.4
		NS	PIM	3.4	1.05	0.9
		EDP	NP	4.2	1.13	2.0
		TS	...	...	1.00	0.0
Fluoxetine		VS	PIM	1.2	1.26	2.5
		NS	POM	3.3	1.05	1.1
		EDP	RP	2.0	1.32	1.8
		TS	...	...	1.00	0.0
Methylphenidate		VS	PIM	0.9	1.48	3.3
		NS	POM	2.6	1.10	1.6
		EDP	PIM	0.4	1.36	1.7
		TS	PIM	2.5	1.12	1.7
Mianserin		VS	PIM	0.6	2.07	3.6
		NS	PIM	0.9	1.21	1.8
		EDP	PIM	0.4	1.38	1.6
		TS	PIM	1.7	1.09	1.0
Lorazepam		VS	RP	11.1	1.03	0.5
		NS	...	...	1.00	0.0
		EDP	PIM	0.3	1.10	0.6
		TS	PIM	0.4	3.60	6.3
Temazepam		VS	RP	7.4	1.12	1.0
		NS	RP	6.7	1.04	0.5
		EDP	PIM	0.3	1.13	0.6
		TS	NP	2.8	1.12	1.0
(c) Pharmaceuticals						
Carbinoxamine		VS	PIM	1.3	1.08	0.8
		NS	PIM	2.3	1.06	1.0
		EDP	NP	5.0	1.14	2.1
		TS	...	...	1.00	0.0
Propranolol		VS	POM	2.2	1.13	1.7
		NS	POM	5.3	1.59	5.0
		EDP	POM	1.2	1.07	0.6
		TS	POM	3.1	1.15	2.3
Phensuximide		VS	RP	1.3	1.11	1.4
		NS	RP	1.2	1.05	0.6
		EDP	NP	0.5	1.10	0.9
		TS	RP	1.3	1.16	1.9
Proglumide		VS	RP	4.1	2.10	3.5
		NS	RP	3.9	2.10	3.9
		EDP	PIM	0.4	1.16	0.7
		TS	RP	2.9	1.16	1.9

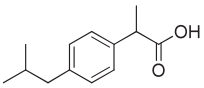
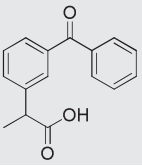
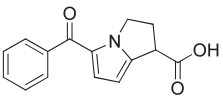
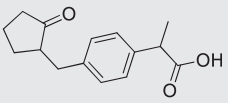
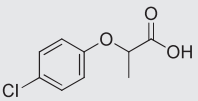
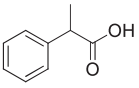
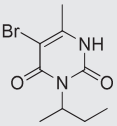
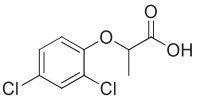
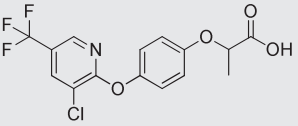
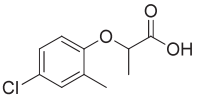
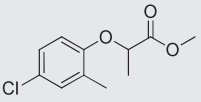
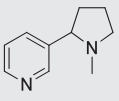
(Continues)

TABLE 1 (Continued)

Name[a]	Structure[a]	CSP[b]	MP[c]	k_1[d]	α[d]	R_s[d]
Hexobarbital		VS	RP	2.0	1.18	1.8
		NS	RP	1.6	1.11	1.4
		EDP	RP	2.5	1.14	1.2
		TS	RP	1.3	1.14	1.4
Warfarin		VS	RP	8.5	1.10	1.5
		NS	RP	9.5	1.04	1.4
		EDP	NP	0.8	1.05	0.5
		TS	RP	3.0	1.32	3.5
(d) Amino acids and derivatives						
Phenylalanine		VS	NP	5.1	1.08	0.6
		NS	...	...	1.00	0.0
		EDP	PIM	0.5	1.20	0.8
		TS	RP	0.7	1.40	2.3
FMOC phenylalanine		VS	RP	0.9	1.07	0.5
		NS	...	...	1.00	0.0
		EDP	PIM	1.0	1.27	1.7
		TS	PIM	0.2	2.33	2.5
4-Nitrophenylalanine		VS	RP	0.6	1.11	1.0
		NS	RP	1.0	1.07	0.6
		EDP	RP	0.8	1.13	0.7
		TS	RP	1.3	1.19	1.4
Kynurenine		VS	RP	0.8	1.98	3.2
		NS	...	...	1.00	0.0
		EDP	RP	1.3	1.40	1.9
		TS	RP	1.0	3.67	8.5
DOPA		VS	...	...	1.00	0.0
		NS	...	...	1.00	0.0
		EDP	RP	0.4	1.50	1.7
		TS	RP	0.5	1.75	2.4
2-Amino-2-phenylbutyric acid		VS	RP	0.3	1.14	0.7
		NS	POM	1.6	1.12	0.6
		EDP	PIM	0.5	1.57	2.7
		TS	RP	0.7	2.12	3.6
(e) Nonsteroidal anti-inflammatory drugs						
Benoxaprofen		VS	...	...	1.00	0.0
		NS	...	...	1.00	0.0
		EDP	PIM	0.9	1.35	2.0
		TS	...	...	1.00	0.0
Etodolac		VS	...	...	1.00	0.0
		NS	...	...	1.00	0.0
		EDP	PIM	0.7	1.17	1.1
		TS	RP	0.8	1.08	0.9
Flurbiprofen		VS	...	...	1.00	0.0
		NS	...	...	1.00	0.0
		EDP	PIM	0.9	1.37	2.1
		TS	RP	3.3	1.12	1.4

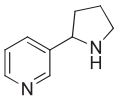
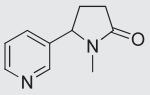
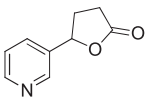
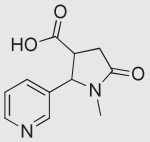
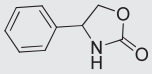
(Continues)

TABLE 1 (Continued)

Name[a]	Structure[a]	CSP[b]	MP[c]	k_1[d]	α[d]	R_s[d]
Ibuprofen		VS	...	...	1.00	0.0
		NS	...	...	1.00	0.0
		EDP	PIM	0.6	1.26	1.4
		TS	RP	3.2	1.15	1.4
Ketoprofen		VS	...	...	1.00	0.0
		NS	...	...	1.00	0.0
		EDP	PIM	0.9	1.31	1.8
		TS	RP	1.7	1.06	0.7
Ketorolac		VS	...	...	1.00	0.0
		NS	...	...	1.00	0.0
		EDP	PIM	1.1	1.10	0.9
		TS	PIM	0.4	2.40	3.5
Loxoprofen		VS	...	...	1.00	0.0
		NS	...	...	1.00	0.0
		EDP	PIM	0.5	1.17	1.5
		TS	RP	2.6	1.19	1.8
(f) Pesticides						
2-(4-chlorophenoxy) propionic acid		VS	...	...	1.00	0.0
		NS	NP	0.3	1.08	0.5
		EDP	PIM	0.9	1.36	2.1
		TS	PIM	0.1	3.64	3.5
2-phenylpropionic acid		VS	...	...	1.00	0.0
		NS	...	...	1.00	0.0
		EDP	PIM	0.8	1.18	1.2
		TS	RP	0.8	1.11	1.1
Bromacil		VS	RP	2.9	1.14	1.6
		NS	RP	2.7	1.04	0.6
		EDP	RP	1.7	1.05	0.6
		TS	RP	2.2	1.18	2.1
Dichlorprop		VS	...	...	1.00	0.0
		NS	...	...	1.00	0.0
		EDP	PIM	0.9	1.37	2.1
		TS	PIM	0.1	1.70	3.5
Haloxyfop		VS	RP	9.6	1.05	0.7
		NS	...	...	1.00	0.0
		EDP	PIM	0.5	1.26	1.4
		TS	PIM	0.1	1.70	3.6
Mecoprop		VS	...	...	...	0.0
		NS	...	...	...	0.0
		EDP	PIM	0.7	1.36	2.1
		TS	PIM	0.1	1.70	2.9
Mecoprop methyl ester		VS	RP	6.6	1.10	1.4
		NS	RP	6.8	1.17	2.0
		EDP	...	...	...	0.0
		TS	...	...	...	0.0
(g) Nicotine and metabolites						
Nicotine		VS	NP	15.1	1.06	0.6
		NS	PIM	0.8	1.81	3.5
		EDP	NP	4.0	1.05	0.6
		TS	PIM	1.6	1.04	0.4

(Continues)

TABLE 1 (Continued)

Name[a]	Structure[a]	CSP[b]	MP[c]	k_1[d]	α[d]	R_s[d]
Nornicotine		VS	PIM	2.6	1.08	1.1
		NS	PIM	10.7	1.19	3.1
		EDP	...	...	1.00	0.0
		TS	...	...	1.00	0.0
Cotinine		VS	PIM	0.3	1.11	0.6
		NS	...	...	1.00	0.0
		EDP	NP	2.4	1.02	0.3
		TS	PIM	0.8	1.12	1.1
5-(3-pyridyl)tetrahydrofuran-2-one		VS	NP	12.2	1.13	1.4
		NS	NP	7.1	1.14	1.5
		EDP	NP	3.0	1.01	0.2
		TS	RP	3.4	1.10	1.6
Rac-(*,*)-4-*trans*-cotinine carboxylic acid		VS	PIM	1.0	1.12	0.9
		NS	NP	5.9	1.33	1.8
		EDP	PIM	1.3	1.39	2.1
		TS	RP	1.0	1.2	1.4
(h) Oxazolidinone						
4-Phenyl-2-oxazolidinone		VS	NP	2.3	1.26	2.5
		NS	...	...	1.00	0.0
		EDP	NP	1.1	1.12	1.4
		TS	RP	3.2	1.79	4.2

Abbreviations: CSPs, chiral stationary phases; EDP, Edman degradation product; MP, mobile phase; NP, normal phase; NS, NicoShell; PIM, polar ionic mode; POM, polar organic mode; RP, reversed phase; TS, TeicoShell; VS, VancoShell.

[a]See Section 2 for sample information.

[b]Core-shell chiral stationary phases were all 100 × 4.6 mm (i.d.): VS, NS, Vancomycin EDP, and TS. See Section 2 for more information.

[c]See Section 2 for MP conditions: PIM, POM, RP, and NP.

[d]See Section 2 for chromatographic calculations of retention factor of the first peak (k_1), selectivity (α), and resolution (R_s).

compounds with two CSPs, and 17 compounds with only one CSP (Table 1). Of the remaining 10 compounds, all had a partial separation ($R_s > 0.0$) with at least one CSP (Table 1). The data from Table 1 is illustrated in Figure 2A. Figure 2A depicts the number of separations in terms of $R_s > 0.0$ (bar 1, red), $0.0 > R_s > 1.5$ (bar 2, blue), and $R_s \geq 1.5$ (bar 3, green) for each CSP. Each macrocyclic glycopeptide-based core-shell CSP was able to separate ($R_s > 0.0$) at least 60% of the 50 chiral compounds (Figure 2A). Edman degradation product had the highest efficacy of the four CSPs, separating 46 of 50 (92%) of the set ($R_s > 0.0$) with only four chiral compounds with $R_s = 0.0$; the three basic amines: trimethylbenzylamine, epinephrine, and nornicotine as well as the nonionizable methyl ester of mecoprop (Figure 2A and Table 1). In Figure 2B, the number of separations with $R_s > 0.0$ (bar 1, red) were distinguished by which chromatographic mode was utilized. Polar ionic mode was the most successful chromatographic mode, utilized overall to perform 40% of the best separations and for each respective CSP: EDP, TS, VS, and NS, 54%, 30%, 38%, and 35% (Table 1 and Figure 2B). Reversed phase was the next most efficient mode, utilized for 31% of the best separations, dominantly for VS and TS, 38% and 48%, respectively. Reversed phase was less useful for NS and EDP, 26% and 13%, respectively (Table 1 and Figure 2B). Normal phase and POM were less utilized as the best mobile phases, only used for 17% and 12% of all the best separations obtained by the four CSPs (Table 1 and Figure 2B).

3.2 | Complementary behavior and best applications

As expected, VS and NS were highly effective for separating basic amines, while TS was more effective for separating acidic compounds, which highlights their complementary behavior. Edman degradation product was most like the teicoplanin chiral selector as it separated most chiral acids, like the amino acids, herbicides, and nonsteroidal anti-inflammatory drugs (Table 1). To

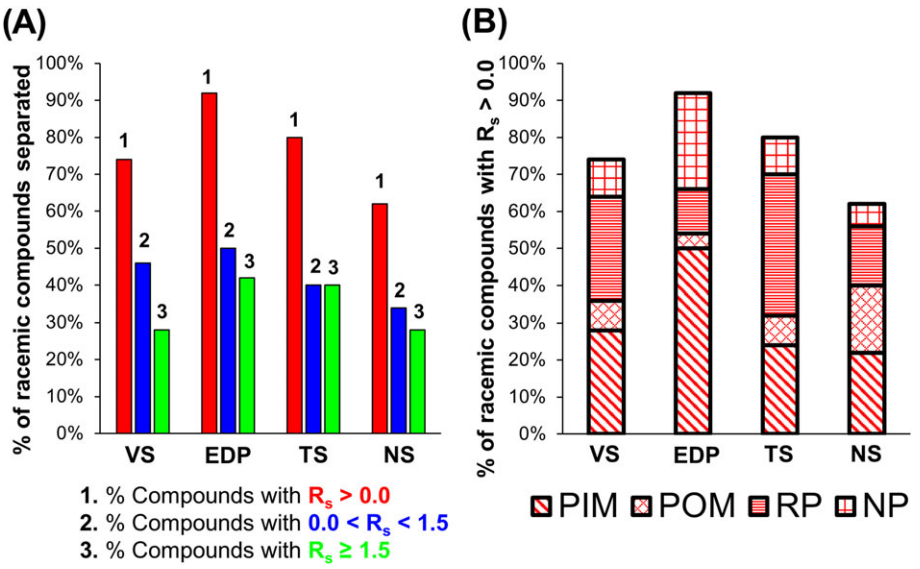


FIGURE 2 Percentage of racemic compounds separated by each macrocyclic glycopeptide-based chiral stationary phase (CSP): VancoShell (VS), Vancomycin Edman degradation product (EDP), TeicoShell (TS), and NicoShell (NS). A, The highest resolution (R_s) for all 50 compounds obtained during screening (from Table 1) by each CSP is indicated by each bar. Bar 1 (red) represents the percentage of racemic compounds with $R_s > 0.0$, while bar 2 (blue) indicates the percentage of racemic compounds with $0.0 < R_s < 1.5$, and bar 3 (green) shows the percentage of baseline separations obtained ($R_s \geq 1.5$). B, Bar 1 ($R_s > 0.0$ during screening from Table 1) from Figure 2A for each CSP is distinguished into the chromatographic modes utilized. From the bottom to the top of each bar, it is divided into polar ionic mode (diagonal lines), polar organic mode (crisscross), reversed phase (horizontal lines), and normal phase (grid). See Section 2 for chromatographic parameters and information

illustrate this, Figure 3 compares the R_s obtained from a selection of 10 chiral compounds between each CSP. Edman degradation product was able to differentiate

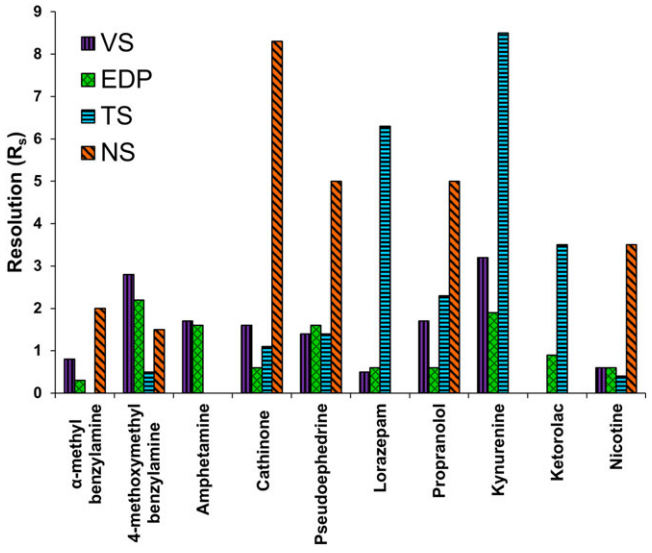


FIGURE 3 Comparison of resolution (R_s) obtained from VancoShell (VS, purple and vertical lines), the vancomycin Edman degradation product (EDP, green and crisscross), TeicoShell (TS, light blue and horizontal lines), and NicoShell (NS, orange and diagonal lines) to emphasize the broad-spectrum recognition of VS and EDP compared with the high chiral selectivity obtained from TS and NS for 10 selected chiral compounds (full data in Table 1). See Section 2 for chromatographic parameters and information

all 10 compounds, exhibiting the broadest spectrum of the four CSPs. However, the most effective selector was not EDP for each of the 10 compounds. TeicoShell was more selective than EDP and had the highest R_s for 19 of the 50 compounds (Table 1). As shown in Figure 3, the R_s was 3 to 10 times higher for TS compared with the other CSPs for the neutral lorazepam and the two acids, kyurenine and ketorolac. Clearly, the acidic enantiomers are most easily recognized by TS. Similarly, when examining the results of VS, it was clearly the most applicable CSP for basic enantiomers. However, certain compounds were more selective to NS and EDP. For example, EDP was the only CSP to separate the nonsteroidal anti-inflammatory benoxaprofen (Table 1). Also, NS was by far the best CSP for cathinone, pseudoephedrine, propranolol, and nicotine with R_s factors 3 to 10 times higher than the other CSPs (Figure 3). Overall, the screening procedure showed that 19, 13, 10, and 8 compounds were best separated by TS, NS, EDP, and VS, respectively (Table 1).

Specific applications of these CSPs based on their complementary behavior are shown in Figure 4. The simultaneous separations of rac-amphetamine and rac-methamphetamine in 5 minutes, rac-pseudoephedrine and rac-ephedrine in 10 minutes, and rac-dichlorprop and rac-haloxyfop in 5 minutes are shown using VS, NS, and TS, respectively (Figure 4A,B,C). Edman degradation product was the most effective CSP for the separation of

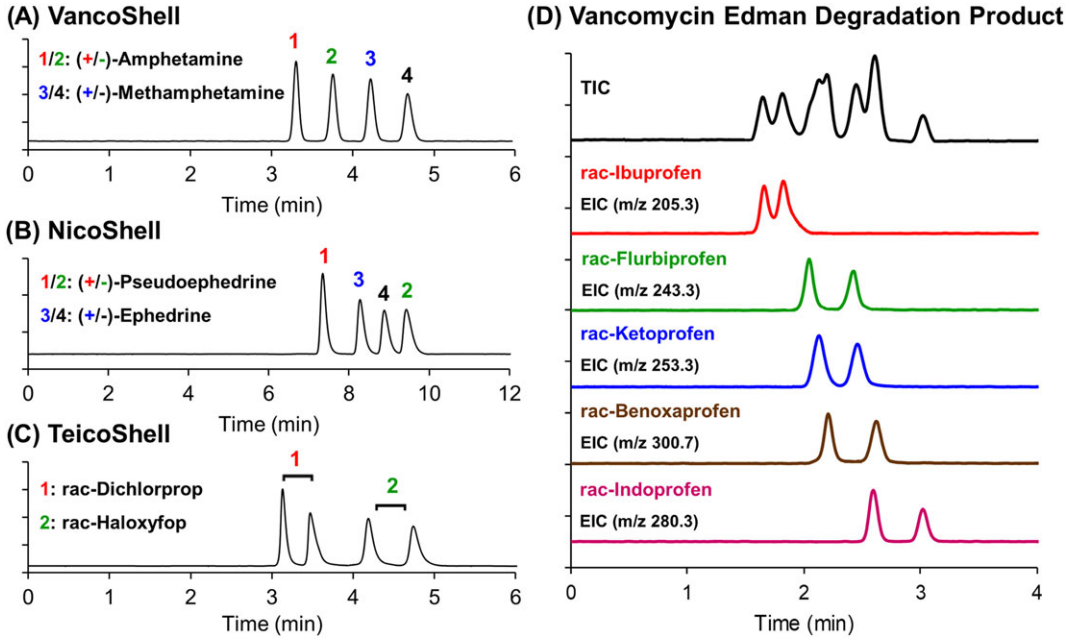


FIGURE 4 Highlighted applications of core-shell macrocyclic glycopeptide-based chiral stationary phases. A, Separation of rac-Amphetamine and rac-Methamphetamine using VancoShell (100 × 4.6 mm [i.d.]) with MeOH-AA-TEA (100:0.2:0.1, v/v/v) at 0.5 mL/min, 25°C, UV 254 nm. (1): (+)-Amphetamine, (2): (−)-Amphetamine, (3): (+)-Methamphetamine, (4): (−)-Methamphetamine. B, Separation of rac-Pseudoephedrine and rac-Ephedrine using NicoShell (100 × 4.6 mm [i.d.]) with MeOH-AA-NH_4OH (100:0.2:0.05, v/v/v) at 0.5 mL/min, 25°C, UV 220 nm. (1): (+)-Pseudoephedrine, (2): (−)-Pseudoephedrine, (3): (+)-Ephedrine, (4): (−)-Ephedrine. C, Separation of rac-Dichlorprop and rac-Haloxyfop using TeicoShell (100 × 4.6 mm [i.d.]) with MeOH-NH_4Formate (pH 3.6; 16 mM) (30:70, v/v) at 0.8 mL/min, 45°C, UV 230 nm. D, Simultaneous LC-MS separation of rac-Ibuprofen (red), rac-Flurbiprofen (green), rac-Ketoprofen (blue), rac-Benoxaprofen (brown), and rac-Indoprofen (pink) using the vancomycin Edman degradation product (100 × 4.6 mm [i.d.]) with MeOH-NH_4Formate (100:0.1, v/w) at 1.0 mL/min, 25°C, UV 230 nm. The total ion chromatogram (TIC) is shown in black, and each profen is shown according to their m/z in the extracted ion chromatograms (EICs). See Section 2 for more information

racemic profens (nonsteroidal anti-inflammatory drugs) with fast analysis times using PIM, which is shown in Figure 4D. The simultaneous LC-MS separation of five profens was performed within approximately 3 minutes, which is shown in the total ion chromatogram, then each profen's m/z extracted in the subsequent extracted ion chromatograms (Figure 4D). This instrument was not optimized for its extra column band broadening, explaining the lower efficiency observed compared with the screening results, such as for rac-ibuprofen.

4 | CONCLUSIONS

The screening procedure used to evaluate the new vancomycin EDP as a chiral selector demonstrated its ability to discriminate the enantiomers of 46 chiral compounds of a set of 50. The other recent modified macrocyclic glycopeptide-based core-shell CSP, NS, was shown to separate some nonionizable and acidic compounds but was most useful for amines, like beta blockers and stimulants. These modified macrocyclic glycopeptides provide examples of complementary behavior with their native analogs, indicating the value and need for investigation of new macrocyclic glycopeptide chiral selectors.

ACKNOWLEDGMENTS

We thank AZYP, LLC, for their technical support for HPLC chiral column technology. This work was supported by the Robert A. Welch Foundation (Y0026) and the French National Center for Scientific Research (ISA-CNRS-UMR5280).

ORCID

Garrett Hellinghausen http://orcid.org/0000-0002-5427-327X
Alain Berthod http://orcid.org/0000-0002-7452-9527
Daniel W. Armstrong http://orcid.org/0000-0003-0501-6231

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How to cite this article: Hellinghausen G, Lopez DA, Lee JT, et al. Evaluation of the Edman degradation product of vancomycin bonded to core-shell particles as a new HPLC chiral stationary phase. *Chirality*. 2018;1–12. https://doi.org/10.1002/chir.22985